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The potential of mini-grafting for large-scale production of *Prosopis alba* clones

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Abstract

With the objective to develop rapid, cost-effective asexual propagation methods for elite clones of *Prosopis alba*, grafting treatments were examined on small (2 mm diameter) seedlings that are easier and more rapid to graft than larger stems. Three techniques were evaluated to seal the wedge graft union (black plastic electrical tape, clear plastic grafting tape, and Parafilm™ as used in biochemical laboratories) and two treatments (black plastic tape and water-soluble mastic) were evaluated to seal the distal end of the scion. These graft union/distal end-sealing techniques were examined under and outside a plastic tunnel with intermittent mist to maintain high humidity. All of the treatments were examined under a 65% shade cloth in which the mean and 95% confidence interval of light intensity was $329 \pm 53\,\mu mol\,m^{-2}\,s^{-1}$ and the mean and 95% confidence interval noon temperatures under and outside the tunnel were $28.8 \pm 1.23^{\circ}C$ and $26.4 \pm 1.19^{\circ}C$, respectively. The maximum percentage grafting of 70% was obtained under the tunnel with a combination of parafilm for the graft union and black mastic to seal the distal end of the scion. Lower successes were obtained from grafts with individual plastic bags outside the tunnel. The maximum rate of $20\,graft\,h^{-1}$ (two people) would represent an additional cost of about $\$33\,ha^{-1}$ for the recommended planting density of $100\,trees\,ha^{-1}$ which would be almost insignificant in the total cost over a 30-year rotation of about $2000. This is the only technique currently available that is sufficiently rapid and reliable for use in establishing commercial clonal plantations of *Prosopis alba*.

Keywords: Agroforestry; Genetic improvement; Nitrogen fixation; Reforestation; Semi-arid

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1. Introduction

In the last 10 years, there has been considerable progress in cloning elite, semi-arid-adapted, nitrogen-fixing trees of the genus *Prosopis* for fast growth for lumber production, pod production and pod quality (Alban et al., 2001; Felker et al., 2001).

Despite considerable advances in rooting of cuttings (Klass et al., 1984, 1986) and tissue culture of Prosopis (Jordan and Balboa, 1985; Batchelor et al., 1989; Green et al., 1990; Ramawat and Nandwani, 1991), there still is no asexual propagation technique for *Prosopis alba* which is suitable for production of massive quantities of clones that would be required for commercial scale plantations. Rooting of cuttings even from rejuvenated greenhouse-grown stock plants is too variable in rooting (Felker, unpublished observation). Grafting has not thought to have been a commercially viable technique for large-scale plantations because the 4–8 mm diameter rootstocks described by Wojtusik and Felker (1993) requires too much expense to grow and graft. Due to the much greater ease in rooting *P. juliflora* and *P. pallida* stem cuttings than *P. alba* (Leakey et al., 1990), commercial production of *P. pallida/juliflora* clones by rooting of cuttings may be possible (Alban, 2001, personal communication).

On a visit to collaborators Harsh and Tewari in Jodhpur, India, in January 2000, we observed their success in grafting small, about 2-mm diameter seedlings of *P. juliflora*. As grafting such small plants offers considerable advantages in (1) reduced time to grow the plant, (2) more flexible and pliable stems and cambium layers and (3) smaller stem areas to cut and shape, we decided to investigate this promising technique for commercial asexual propagation of *P. alba*.

2. Materials and methods

The grafting trials were conducted at the Fernandez Forestry Experimental Station of the Catholic University of Santiago del Estero, Argentina which is at 27°S latitude. The experiment began on October 12 2000 (spring in southern hemisphere) and continued for 100 days. All the treatments were located under 65% shade cloth as our previous grafting work (Felker et al., 2000) indicated this light regime was preferred.

A modified randomized complete-block, split-plot design was used. The two major treatments were grafts within a microtunnel or grafts covered with individual plastic bags. Within the main treatments, there were four treatments to compare combinations of sealing the graft union and the distal part of the graft scion. Each of the four treatments had eight grafts per replicate (32 per graft union/distal treatment, tunnel or bag treatment) resulting in a precision of measurement of grafting success of 12%. Means and 95% confidence intervals (based on four means of eight grafts per replicate and not the 32 total grafts per treatment combination) were calculated using Excel and indicated in the graphs. These confidence intervals permit the reader to gauge the significance of the treatments and to observe the substantial differences in variability among treatments that would not be observed by a common pooled confidence interval. Due to the simplicity of the experiments

and the fact that most of the survivals were in the 20–80% range, it was not deemed necessary to arcsine transform the percentage data.

The same wedge-type graft (Wojtusik and Felker, 1993) was used for all treatments. Our previous experiments indicated that it was critical to tightly wrap the distal end of the scion with black electrical tape to prevent desiccation and rapid death of the scion. As wrapping the distal end of 2 mm diameter seedlings is tedious, we compared tape for sealing the distal end to use of a water-soluble, black mastic that is known in Argentina as Protex and used for undercoating automobiles. With the objective of increasing the rate of grafting, we compared traditional local available clear grafting tape, black electrical tape and Parafilm™ as normally used in biochemical laboratories. Parafilm has the advantage of being moldable and thus easy to seal delicate graft unions. The rate of grafting was for two people working together. The times for the grafting did not include the time to place and seal the plastic bags over the grafts.

Four separate tunnels were each 1-m wide, about 0.5-m high and contained mist nozzles operating with a pressure of 2 MPa that were manually activated once a day for 10 min. On the 70th day after the grafts were made, when the unions were well established, the tunnels were removed.

In summary, each of the four blocks had a small tunnel containing 32 grafts that were kept moist with mist heads and 32 grafts in individual plastic bags with no misting. Due to the need to quickly place the grafted plants without bags in a high-humidity environment, the plants in the tunnels were grafted on one day and the plants for plastic bags on the second day. However, within each day, all plants within a block were grafted at the same time. Due to increased skill as the grafting progressed, the rate of grafting was greater on the second day when grafting the plants with individual plastic bags. Thus, a block effect of increased rate of grafting was noted on the second day. As no improved seed of *P. alba* has been produced, a mixture of seeds from local trees was used to produce seedlings that were grown in 6-cm diameter 18-cm tall clear plastic bags. The seeds were sown and germinated in the latter part of February (beginning of fall), but the seedlings did not grow in the winter and had just begun to grow again in the spring when they were grafted. The mean height and diameter of the seedlings and the rate of grafting is presented in Table 1. About every 2 weeks, the heights of the grafted sprouts and the mortality of the grafts was measured.

The temperatures were taken at 1200 in each of the four blocks under the plastic and outside the blocks, but under the half-shade. On about 10 dates, the temperatures were measured at 0900, at 1200 and at 1700. On the days with the highest temperatures, the maximum temperatures were recorded at 1700 and were often 4°C higher than the 1200 readings. Light-intensity measurements were taken daily at noon with a Licor Model 185B light meter.

3. Results

As can be seen in Table 1, there was considerable uniformity in the height and diameter of the seedlings within the tunnel or plastic bag treatments. However, the

Table 1
Prosopis alba rootstock seedling characteristics used in the trials of mini grafting

Treatments	Grafts under tunnel					
	Grafts/h		Height (cm)		Diameter (mm)	
Graft union/distal end	Mean	95% CI	Mean	95% CI	Mean	95% CI
Blacktape/black tape	14.6	0.81	26.1	1.76	2.1	0.15
Blacktape/mastic	15.8	0.99	25.7	2.87	2.0	0.13
Cleartape/mastic	16.2	1.76	25.9	1.77	2.0	0.17
Parafilm/mastic	18.2	0.69	26.0	1.42	2.0	0.09
Grafts out of tunnel in individual plastic bags						
Graft union/distal end	Mean	95% CI	Mean	95% CI	Mean	95% CI
Blacktape/black tape	18.6	4.5	22.5	1.9	2.1	0.2
Blacktape/mastic	19.1	2.6	22.6	1.3	2.1	0.2
Cleartape/mastic	20.0	4.0	24.1	2.4	2.0	0.0
Parafilm/mastic	20.3	2.9	23.4	2.6	2.0	0.1

Each of the graft union/distal end combinations had eight grafts per replicate, four replicates, and two treatments (under and outside the tunnel) for 64 grafts per graft union/distal end treatment.

seedlings for the plastic bags out of the tunnel were slightly shorter, although they were of virtually the same diameter. As noted in Section 2, on the second day, the rate of grafting increased with successive blocks resulting in greater confidence intervals and an overall more rapid rate of grafting than under the tunnel.

As judged by the confidence intervals, use of the mastic on the distal end significantly increased the rate of grafting as opposed to sealing the ends with black tape (in the tunnel). However, on the second day, when all rates of grafting increased with experience, the confidence intervals increased and the use of mastic was not significantly different from use of the tape. In a comparison of the rate of sealing the unions, the parafilm tape was more rapid than either the black or clear plastic tape.

The noon daily light intensity shown in Fig. 1 illustrates the considerable day-to-day variation in light intensity due to the onset of the rainy season and partially cloudy weather in December. The mean and 95% confidence interval of light intensity of $329 \pm 53\ \mu mol\ m^{-2}\ s^{-1}$ was similar to the means of 270–339 $\mu mol\ m^{-2}\ s^{-1}$ reported by Felker et al. (2000) for grafting *P. alba* onto *P. ruscifolia*.

The noon daily temperatures for the first 70 days in and out of the tunnel in Fig. 2 illustrate the important point that when such a tunnel is placed beneath a 65% shade cloth, 10°C rises in temperatures, such as occurs in unshaded greenhouses without cooling, does not occur. The mean and 95% confidence intervals for the temperatures inside vs. outside the tunnel were 28.8 ± 1.23°C and 26.4 ± 1.19°C, respectively.

The percentage and 95% confidence intervals for the survivals in Fig. 3 show the dramatic improvement in grafting success under the tunnels. Treatments in which the means and confidence interval bars did not overlap were significantly different at the 95% probability level. Under the tunnel, the combination of parafilm for the

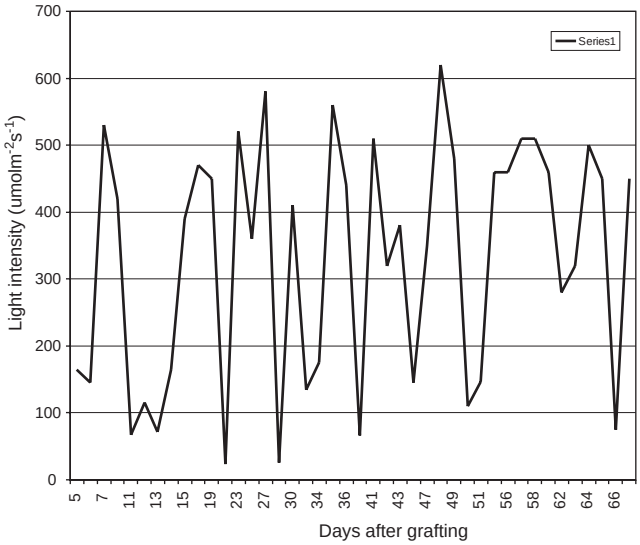

Fig. 1. Daily noon light-intensity measurements under the 65% shade cloth in Fernandez, Argentina, as a function of days after grafting 2-mm diameter *P. alba* seedlings.

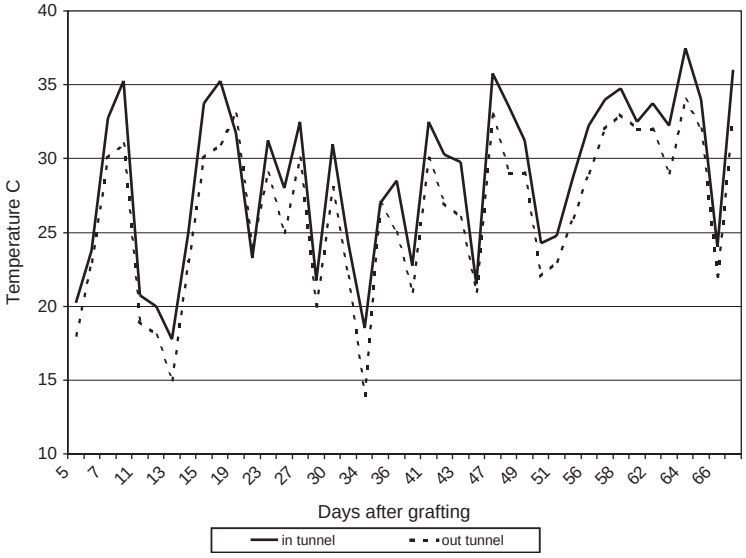

Fig. 2. Daily noon air temperatures under and outside the tunnel under the 65% shade cloth in Fernandez, Argentina, as a function of days after grafting.

graft union and black mastic to seal the distal end of the scion had the greatest percentage of 70% and was significantly different from the treatment with the black tape/mastic combination. The grafting success was much lower in the individual

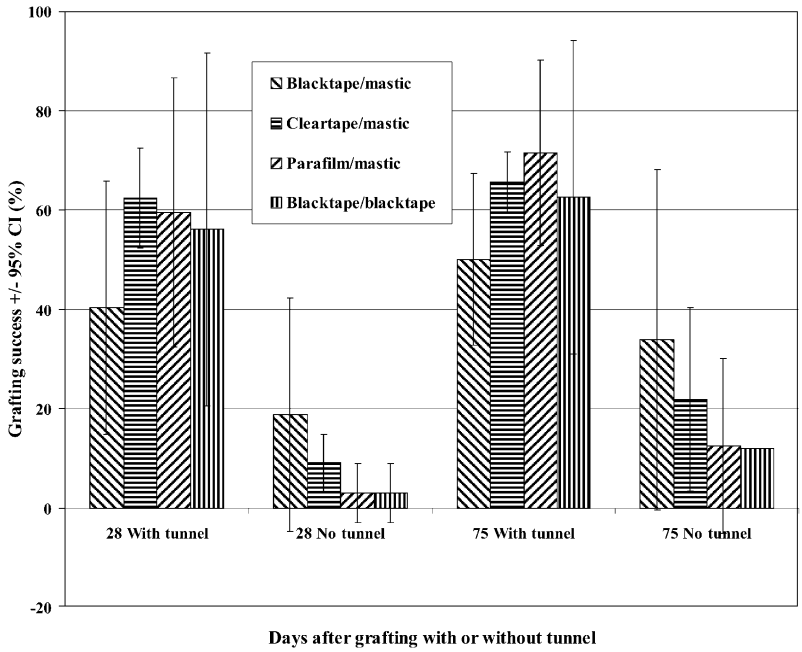

Fig. 3. Mean and 95% confidence intervals for percentage grafting success of *P. alba* with or without the tunnel as a function of graft union/distal end-sealing techniques of black tape/mastic, clear tape/mastic, parafilm/mastic and blacktape/black tape.

plastic bags outside the tunnel and the ratio of the confidence interval to the mean was very much greater than for the treatments under the tunnel. The ranking of the treatments for percentage survival of the grafts was also different from under the tunnel, but this may be due to chance as the confidence intervals were very large.

The growth of the grafted shoots (means of only live shoots) in Fig. 4 illustrate the more rapid growth under than outside the tunnel. Surprisingly, for the grafts that survived, the heights at the end of 90 days were not very different from the grafts under the tunnel (data not presented). As opposed to substantial growth differences within the treatments outside the tunnel, the means under the tunnel were virtually identical. If resources were not available to graft under a tunnel, it would appear that the black tape/mastic combination would be preferred as it gave the highest survival (however, which was less than half of the maximum survival obtained under the tunnel).

4. Discussion

Relatively little is known about the light-intensity requirements for successful grafting or rooting of cuttings. Klass et al. (1984) found that high light intensities were absolutely critical to rooting of *P. alba* cuttings as only 9% rooting was

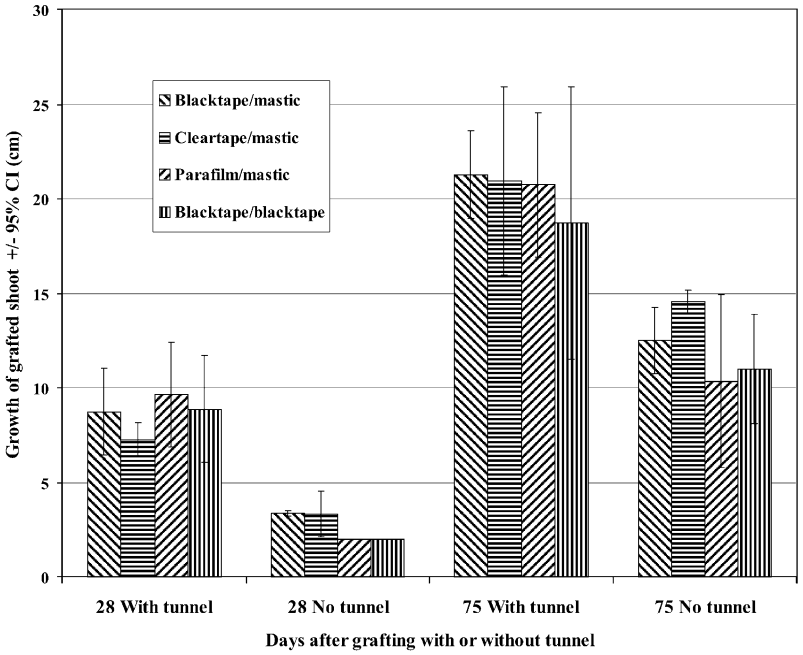

Fig. 4. Mean and 95% confidence intervals for growth of grafted scion of *P. alba* with or without the tunnel as a function of graft union/distal end sealing techniques of black tape/mastic, clear tape/mastic, parafilm/mastic and blacktape/black tape.

obtained at 150 $\mu mol\,m^{-2}\,s^{-1}$ but 69% rooting was obtained at 520 $\mu mol\,m^{-2}\,s^{-1}$. Felker et al. (2000) found that grafts of *P. alba* onto *P. ruscifolia* had about 80% success in the second grafting period when the mean noon light intensity under a black plastic shade was only 127 $\mu mol\,m^{-2}\,s^{-1}$. The mean noon value of 329 $\mu mol\,m^{-2}\,s^{-1}$ we measured is intermediate between the minimum requirement for rooting of cuttings and grafting.

We believe it will be possible to increase the maximum grafting rate of 20 h^{-1} we obtained with a two-person team since workers in nearby nurseries graft nearly 200 avocado seedlings per person per day (J. Palacios, 2000, personal communication). With a daily production of 150 seedlings per day by a two-person team, and Argentine semi-skilled labor costs of \$US 25 day^{-1}, the cost to graft a seedling would only be \$0.33 each. At the suggested planting density of 100 trees ha^{-1}, the \$33 ha^{-1} added initial investment would be insignificant over the life of a 30-year rotation, whose total estimated costs have been estimated to be about \$2000 (Felker, 2000).

We have recently cloned 12 elite *P. alba* trees from a progeny trial that were selected for rapid growth, high pod production and superior pod sensory characteristics (Felker et al., 2001). Unfortunately, despite 2 years of research, it has not been possible to obtain high rooting percentages of these clones and there is great variability in the percentage rooting of these clones. In contrast, both *P. pallida* clones developed in Peru (Alban et al., 2001) and the very closely related *P. juliflora*

(Leakey et al., 1990) are much easier to root and probably commercial plantations of *P. pallida* will be possible using rooted cuttings. Thus, the grafting technique reported here offers a commercially realistic alternative to the propagation of *P. alba* by rooted cuttings. It will be important to develop superior rootstocks for the existing elite budwood in the form of cuttings from easy-to-root clones or seed from clonal seed orchards.

Stem cankers resulting from fungi of the genera *Lasiodiplodia* and *Pestalotiopsis*, which are easily controlled by benomyl and captan, have been shown to cause necrosis and death of *Prosopis* species when exposed to high air humidities (Lesney and Felker, 1995). We have observed stem necrosis in both the individual plastic bags and under the tunnels but have not confirmed the identity of the casual organisms. In this regard, the tunnel has a significant advantage in that it is easy to open the tunnel for short periods to apply fungicides. While benomyl is highly effective in controlling these stem fungal pathogens, it has been shown to be inhibitory to root formation from stem cuttings (Klass et al., 1986).

These seedlings were only 2 mm in diameter despite the fact that they were 8 months old because they were sown in small containers out of doors in late summer (February) and grafted in the beginning of spring. In a greenhouse environment with larger root containers, a 2-mm diameter grafted seedling could be produced in 45 days. In another 120 days these grafted plants would have a grafted height of 50 cm and would be ready for planting in the field. Thus, the total time from seed to a grafted plant ready for the field would be about 165 days.

In summary, the recommended grafting technique would be grafting under a tunnel with the use of parafilm for the graft union, as it is more rapid and yielded higher grafting success, and the water-soluble black mastic for the distal end of the scion. The current grafting rate of 150 grafts per day using two people would result in a grafting cost of about $0.33 each, which would be a minor cost over the 30-year life of a *Prosopis* timber/pod plantation. This is the only technique currently available that is sufficiently rapid and reliable for use in commercial clonal plantations of *P. alba*.

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