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Genetic and agronomic improvement of quandong

A report for the Rural Industries Research and Development Corporation

by Ben Lethbridge and Barbara Randell

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Foreword

The quandong industry is moving from wild-picked to orchard derived fruit with selected varieties. Development of the native Australian quandong to a well managed orchard tree still requires much research – its unusual parasitic habit, in particular, seems unique amongst horticultural fruit crops.

A better understanding of orchard management and production systems will be critical to industry success. Research into genetics, agronomy, orchard management, harvesting and post harvest handling of the product is all necessary.

This publication discusses some innovative projects addressing some of these aspects of quandong production. This research will help speed the development of this unique Australian native food.

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Simon Hearn
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Ben Lethbridge: The financial support of the Rural Industries Research and Development Corporation (RIRDC) and the Australian Quandong Industry Association.(AQIA) is gratefully acknowledged. Thanks to the Port Augusta Council for having the foresight to realise the value of the quandong to the economic development of the region. The technical help of Bernie Haas and other staff at the Australian Arid Land Botanic Gardens (AALBG) has been most useful. Much gratitude to Perce Prenzel for the gift of quandong seed and to Graham and Iris Herde for their hospitality and useful discussion.

Abbreviations

AALBG	Australian Arid Lands Botanic Gardens
ANOVA	ANalysis Of VAriance
AQIA	Australian Quandong Industry Association
Arg	Acacia argyrophylla
Bru	Acacia brumalis
Cal	Acacia calamifolia
CALM	Conservation And Land Management. West Australia
CSIRO	Commonwealth Scientific Industry Research Organization
Hak	Acacia hakeoides
Hem	Acacia hemiteles
LSD	Least Significant Difference
Mur	Acacia murrayana
RIRDC	Rural Industries Research and Development Corporation
Riv	Acacai rivalis
Vic	Acacia victoriae

Contents

Executive Summary

The quandong (*Santalum acuminatum*) industry is a significant component of the native food industry. A close relative is the sandalwood (*Santalum* species), current research in this product is offering the potential for managed plantation style wood production and for the quandong, efficient orchard design offers a reduced burden on unsustainable wild fruit harvest.

The quandong being a partially root parasitic plant, is able to obtain water and nutrients from the roots of near by plants by attachment of sucker like appendages from quandong roots. The industry is currently devoid of sound scientific evidence for appropriate hosts. A most obvious choice of host would be leguminous plants, which includes acacias, having the ability to fix atmospheric nitrogen in its own roots amongst other qualities. Studying this acacia/ quandong association offers many potential benefits for the agronomy of quandong and the ability to produce another native food crop (wattle seed) in the same orchard, thereby diversifying farm production even more. Eight species of semi arid acacia recognised in the native food industry for actual or potential wattle seed production were compared for their ability to support the growth of quandong in an irrigated area of the Australian Arid Land Botanic Gardens at Pt Augusta, South Australia. *Acacia victoriae* was the best of these, and coincidentally is currently recognised as the major source of wattle seed to the native food industry. Direct seeding quandongs adjacent to dripper fed acacia plants would appear to be a robust system for the introduction of quandongs in the field requiring little seedling protection. However, significant care must be taken of the acacia plant to maintain the host parasite complex in a healthy state. There is still some way to go for efficient fruit and seed production and selection of appropriate forms of wattle and quandong will be a necessary step towards this goal.

Understanding the breeding systems of plants is critical to the development of rational plant improvement strategies. Analysis of arid zone plants has shown in some instances the genetic character of polyploidy (multiple sets of the genetic units), may well occur in quandong. If this is the case then it offers some real opportunities for short cuts in the breeding and selection of quality quandongs. The current study examines some features of herbarium specimens of quandong at the macro and microscopic scale for evidence of polyploidy. Unfortunately in this case, no evidence for polyploidy was found, but for wild populations at least, some evidence for high pollen sterility was noted.

1. The value of quandong and allied industry research

1.1 General introduction

The importance of the fruit of *Santalum acuminatum* (quandong), and seed from acacia species (wattle seed) to the native food industry is probably best highlighted by being featured by Australia Post in the 'bush tucker' series of postage stamps released on September 3rd 2002, the year of the outback.

The quandong (*Santalum acuminatum*) is an Australian native tree which produces a red to yellow, dry textured, tart fruit, a popular ingredient in jams and pie fillings in rural areas where the tree grows wild. The fruit is ranked in the top ten of commercially acceptable foods in the native food industry (Graham and Hart 1997). The industry has been conservatively rated at 0.7 to 1.3 million dollar industry (farm gate estimate) (McKinna, 2002, Gordon Mills 2001). Quandong is a close relative of sandalwood. The sandalwood industry, which in Australia consists of harvesting timber of *Santalum album, lanceolatum* and *spicatum,* is of high economic value (McKinnell 1991). The indirect benefits of research into quandong to the sandalwood industry are rarely factored into the equation.

The quandong industry is slowly moving from wild picked to orchard derived fruit with selected varieties. Research into gaining a better understanding of orchard management and production systems will be critical to industry success. Issues covering genetics, agronomy, orchard management, harvesting and post harvest handling of product will need to be addressed. An important issue to investigate is the role of the host plant and selecting desirable hosts for the partial root parasite. (McKinna 2002, Hele 2001)

Wattles (*Acacia* species) need no introduction to the Australian public, the golden wattle representing the national floral emblem of this nation. The wattle seed industry is also another 'top ten' native food (Graham and Hart 1997) and rated conservatively as a $72,000 to $144,000 industry (farm gate prices) (Simpson and Chudleigh 2001,Graham and Hart 1997). *Acacia victoriae* is currently the most important wattle seed used in the Australian Bushfood industry and most is harvested from the wild (Maslin 1998) . Leguminous acacias also have many other potential uses such as flowers and cut foliage, speciality timbers, tannins and gum arabic etc. (Sedgley and Horlock 1997,Simpson and Chudleigh 2001)

The following two, small, innovative projects, Detecting polyploidy in herbarium specimens of Quandong (*Santalum acuminatum*) by Dr. Barbara Randell and Integrated wattle (*Acacia* sp.) and quandong (*Santalum acuminatum*) orchards by Dr. Ben Lethbridge are described. Under the general title of 'genetic and agronomic improvement of quandong' they form significant initial steps in research towards a vibrant quandong industry and native food industry in general.

1.2 References

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2. Integrated wattle and quandong orchards

by Ben Lethbridge

2.1 Introduction

The quandong fruit (*Santalum acuminatum*) and wattle seed from acacia species occupy significant niches in the fledgling native food industry. (Graham and Hart 1996, Ahmed and Johnson 2000). Acacia species are showing significant promise in the sandalwood industry as host plants, (Brand 2001, Radomiljac 1999a, Woodall 2002) with the expectation that leguminous species could also provide valuable timber products and beneficial nature conservation.

A survey by Maslin et al (1998) identified 47 species of acacia which have potential for cultivation in the southern semi arid regions of Southern Australia as a source of seed for human consumption. There has been a small but increasing demand for wattle seed in the native food industry and is part of investigations (all be it minor) into the commercial output in the broadscale planting of acacias to combat dryland salinity (Simpson and Chudleigh 2001). The current study is extending the theme of utilising the multipurpose nature of leguminous acacias as host plants for quandong fruit production.

2.2 Materials and methods

(1) The site

Within the research area of the Australian Arid Land Botanic Gardens is a chenopod heathland (predominantly *Atriplex* and *Maireana* species, these were retained) of deep alkaline (pH 9.5) loam of zero inclination. The area had been previously deep ripped (1992). On a grid pattern of 6 metres by 4 metres were established approximately 22 plants each of 8 species of semi arid, native food acacia species. Each species of semi arid acacia (Table 1) were planted as two rows of 11 plants from tube stock grown on site. They were irrigated with single drippers (8 litres per hour) at the base of each acacia for 2 hr., twice a week (32 litres per week). *Acacia papyrocarpa* , *victoriae* and *Santalum acuminatum* occur naturally on the site (outside the irrigated area).

(2) The species

Eight acacia species were selected from the list prepared by Maslin (1998) and suitability for the site.

Acacia species	Source
A. murrayana	70 km. West of Cobar (AALBG collection)
A. victoriae	Kenmore Park SW (thornless) (AALBG)
A. rivalis	RG 554 K (Australian Bush products)
A. brumalis	CALM D1001
A. calamifolia	44 Km. West of Yunta (AALBG)
A. hakeoides	Quorn (AALBG)
A. hemiteles	CALM N98177
A. argyrophylla	Carrieton (AALBG)

Table 1. Acacia species used in this study and the seed source.

(3) Procedure

In September 1999, the acacia seeds were germinated and transplanted to large forestry tubes. At age seven months the acacias were planted in to the field with a handful of slow release Osmocote and mulching. There was no further addition of fertiliser to the site. In April 2001, two germinated kernels of quandong (orchard derived seed, Whyalla, South Australia) were direct seeded within ten centimetres of each dripper. . The most economic and efficient method for establishing *S. spicatum* is by direct seeding. (Brand and Jones 1999) Quandong heights, stem widths at 10 cms (quandongs aged 2 years)., acacia heights and widths were recorded.

(4) Quandong germination
The shells from approximately 600 selected quandong seed were removed by placing the micropyle of the seed directly against vice jaws and squeezing gently. After removal of the parchment layer the kernels were surface sterilized in 5% household bleach for 30 minutes and rinsed repeatably with cooled boiled rainwater until the smell of bleach was no longer present. The seeds were then rinsed once more with distilled water and soaked over night in 1in 100 dilution of a commercial preparation of gibberelic acid (Grocel GA, 100g/litre gibberelic acid, ICI Australia). The treated kernels were placed on moist sterile vermiculite in self sealing plastic bags and incubated at 20 degrees C. Germination commenced at day 10 and continued for approximately another two weeks. When the root radicle was about 1cm. in length it was stored at 4 degrees C on moist vermiculite for a period of up to a week.

(5) Statistics
Quandong heights and stem diameters were compared using One way ANOVA with Least Significance Difference (LSD) tests, trends were assessed with simple correlation estimates (r =) and two treatment experiments were compared with two tail T tests. Where not specifically mentioned a 5 percent level of significance was used.

2.3 Results

2.3.1 Quandong growth and survival associated with each acacia species

It is evident, even by casual assessment of the growth parameter measurements made (Table 2, Figure 1, 2, 3.), that *Acacia victoriae* would appear to be a superior host for quandong growth when compared to the seven other selected native food acacias chosen for the AALBG research area. Similiarly, although perhaps less importantly *Acacia hakeoides* was the worst performer (Table 2.) For quandongs aged two years little difference could be statistically deciphered from the remaining six acacia species (Table2, Figure 1,2).

Quandong survival (at year 1, April 2002) in the current trial from germinated seed was 53% +/- 4.5 (mean +/- standard error) across the eight species (table 2), (50% for *Acacia victoriae* and *Acacia murrayana*).Comparison of quandong heights and stem diameters of two year old quandongs planted near each acacia species shows a strong linearity of response (r=0.976) (Figure 3).This result would suggest that the host species had little effect on influencing the growth form of the quandong despite differences in growth form of the acacia.(figure 6) For example the selected provenance of *Acacia murrayana* is a tall upright species and *Acacia victoriae* is a multistemmed rounded plant (statistically significant differences between height and width at one year of age for both species (appendix 2)) potentially offering much more sun and wind protection in the initial stages of growth but this did not appear to affect establishment rates or final growth form of the quandong.

Figure 4 quite clearly demonstrates that the growth rate as measured by length and width of branches of acacia (the other seven species show similar plots, see appendix 2) has little influence on the growth rate of the quandong. The predominant growth period for the selected acacias species in year 3 (*A. victoriae* is shown here) is from April 2002 to October 2002 and much less so from October 2002 to April 2003 and yet the juvenile quandongs growth appears quite linear throughout the whole year.

	Apr.2002	LSD	Oct. 2002	LSD	Apr. 2003	LSD	% survival Apr.2002
vic	72.6	a	98.2	a	125.4	a	50
arg	52.3	b	74.7	bc	107.2	b	79
mur	46.4	b	68.6	bc	106.2	b	50
riv	40.1	b	62	cd	94.3	bc	35
cal	44.6	b	66.2	cd	94.3	bc	53
hem	50	b	81	b	93.3	bc	57
bru	38.3	b	58.2	cd	75.9	cd	37
hak	41.2	b	52.6	d	72.9	d	53

Table 2. Average quandong heights (cm.) of 1 year old (April 2002), 1.5 year old (October 2002) and 2 year old (April 2003) grown near the indicated acacia species. The same letter following each score indicates that they are not significantly different by LSD (p=0.05). Each set of values (by date) should be rated independently. The column on the right indicates the quandong survival rate from germinated seed at one year old.

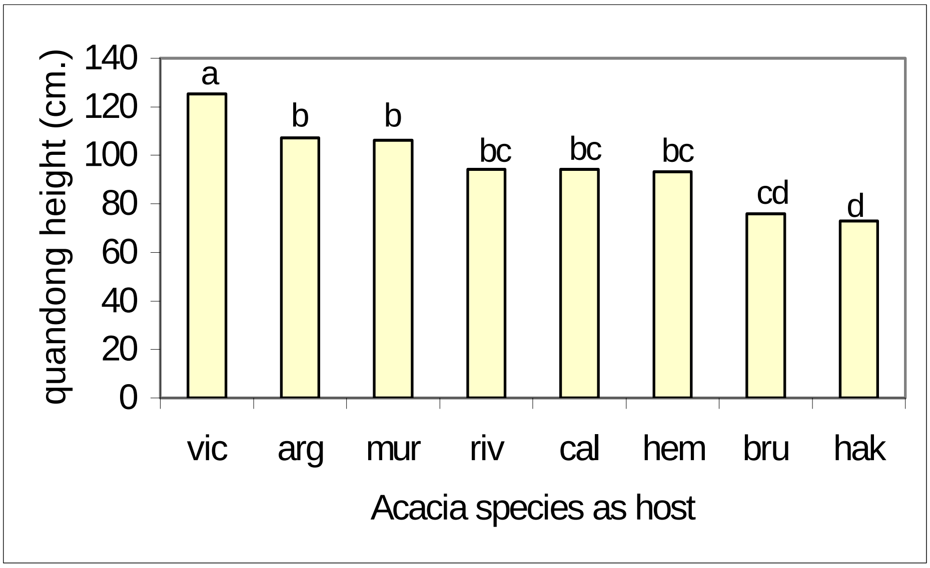
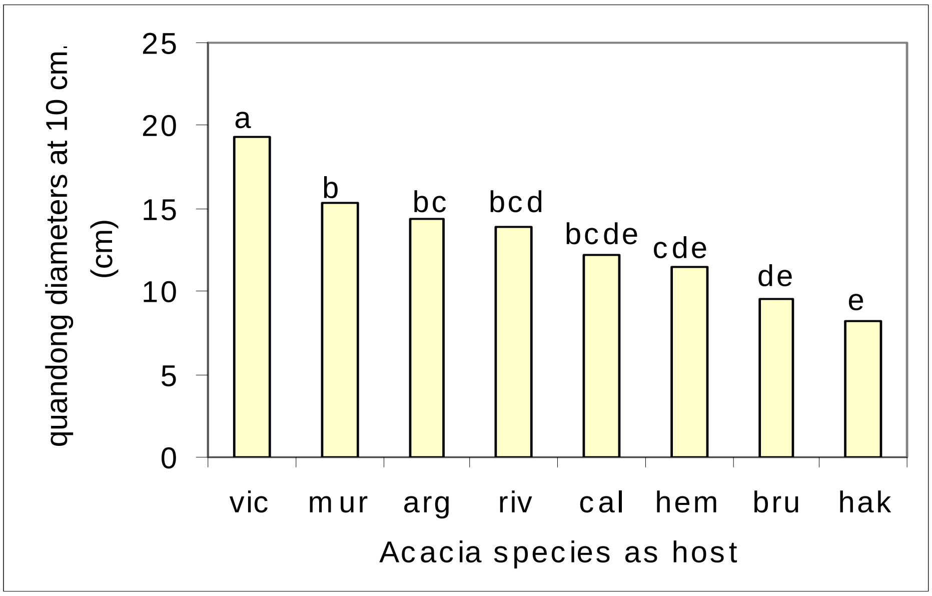


Figure 1. Average quandong height (cm.) at current age 2years (April 2003) planted as germinated seed next to the indicated acacia species, current age 3 years (April 2003). Same letter above column indicates that they are not significantly different by LSD (p=0.05)

Figure 2. Average stem diameters at 10 cm. above ground level for quandong at current age 2 years



(April 2003) planted as germinated seed next to indicated acacia species, current age 3 years (April 2003). Same letter above column indicates that they are not significantly different by LSD (p=0.05)

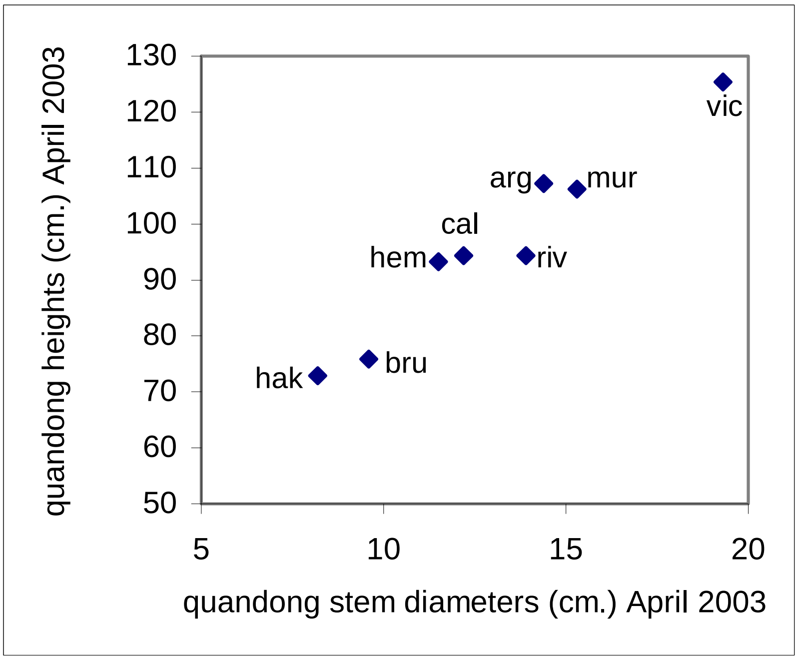
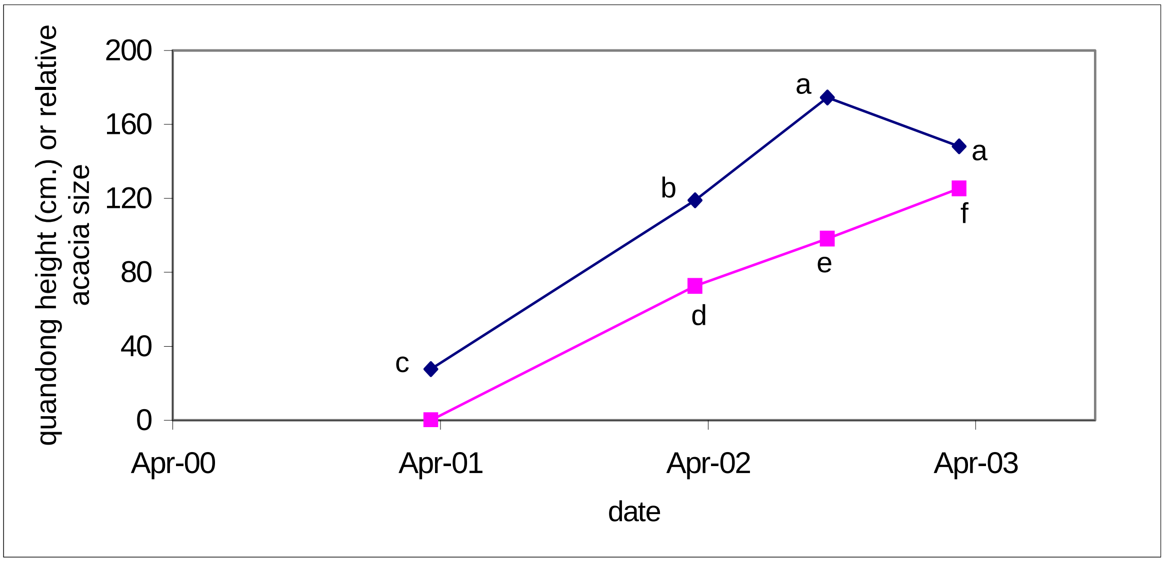


Figure 3. Replot of figures 1 and 2, showing linearity (r=0.976) of average stem diameter at 10 cm. and average height of quandong at age 2 years. Host acacia species to which the quandongs were planted near are indicated.

Figure 4. The lower plot shows the average height of quandong planted near *Acacia victoriae* at the



times indicated, the upper plot is an estimate of average *Acacia victoriae* size calculated as average Height multiplied by average Width for each measurement date divided by 100 (=H x W /100). The same letter associated with each point indicates that they are not significantly different by two tail T test (p=0.05).

Figure 5: *Santalum acuminatum*, age 2 years, growing with *Acacia victoriae*, age 3 years
Date: April 2003
Location: Australian Arid Lands Botanic Gardens Research Area, Pt. Augusta, South Australia

2.4 Discussion

Acacia victoriae shows significantly better growth characteristics for the first two years compared to the other seven selected species at this site. From a commercial point of view this is a good result as *Acacia victoriae* is currently the most important wattle used in the Australian Native Food Industry (Maslin 1998) and is likely to continue so, given its potential dual role. Quandong growers have used this combination because of its native food association and this selection now has a sound scientific basis for its continued use.

2.4.1 Why is *Acacia victoriae* a relatively good host?

A major distinguishing significant feature of *Acacia victoriae* is its high salt tolerance (Maslin 1998). *Santalum acuminatum* is classified as a salt tolerant plant. (Walker 1989) Hence this combination may have value in salt land reclamation. Neither species has yet been recognised for this purpose (Marcar 1995).

It is reasonable to assume that studies with other combinations of acacia/ santalum may have some predictive value in species selection. A host plant pot study by Radomiljac (1999a) of *Santalum album* included *Acacia ampliceps* which has high salt tolerance (Marcar 1995) and out performed *Acacia trachycarpa* as a host for tropical sandalwood. Another pot study by Byrne et al (2002) used the highly salt tolerant *A. cyclops* (Marcar 1995) but could not recommend this species as a good host.

Acacia brumalis has sometimes been found growing in moderately saline soils (Maslin 1998), in this study this species did not stand out as a host for quandong. Similiarly *A. saligna* is often found on moderately saline soils (Marcar 1995, Maslin 1998). This species in a similar study to this (although non irrigated) in WA (Brand et al 2001) was found to be a very good primary host for *Santalum spicatum*, when compared to *A. acuminata, hemiteles* and *microbotrya*, but this may be attributable to the fast growth rate of this species. Nevertheless, combinations of host and quandong with revegetation value in saline areas can only be considered a useful addition to farm management practices. The attribute of salt tolerance in plants is often associated with water conservation strategies.

Little or no research has been done at this stage into the rate of water use of acacia species (Simpson and Chudleigh 2001). Species with high water use rates will perpetuate a less favourable water potential gradient from host to parasite (Radomiljac 1999b, Byrne 1998) with consequent reductions in growth. A study by Brand et al (1999) concluded that the faster growth rate of *S. spicatum* near *A. acuminata* did not appear to be due to greater access to water, especially during the summer period. This study compared *Acacia*, *Allocasuarina* and *Eucalyptus* species, where nutritional differences may be more significant. An alternative theory is that *Acacia victoriae* supplies the correct balance of nutrients to the quandong. *Acacia victoriae* is both iron and phosphate efficient (Handreck 1991) which may have some significance to the current study. There was no evidence for nutrient deficiency or toxicity by visual leaf inspection (see Barrett et al 1993) in this current study for any of the quandongs associated with the eight acacia species, however the possibility cannot at present be excluded. Host: parasite interactions are clearly complex and empirical data will always be a valuable means of assessing species as host plants. Comparison of water use rates and perhaps nutritional variables may offer a glimpse into the mechanism as to why *A.. victoriae* performed well as a host for quandong in this trial at this site.

2.4.2 Other host effects. Shading?

Contrary to Woodall et al (2002) and Barrett et al (1994) there was little evidence for protection through shading of the seedling *Santalum* by the acacia plant. The growth form of quandong (heights vs stem diameter) or quandong survival rates fails to correlate well with the expected protection capacity of each acacia species. The observation that one of the specimens of *Acacia calamifolia* was a prostrate form and produced two seedlings of average height and the 50% survival rate of quandong with the clearly different growth forms of *Acacia victoriae* and *murrayana* would corroborate such a conclusion. A possible explanation is the use of drippers as the artificial water source used in this experiment. The host acacia root concentration would be expected to be high in the wetting zone of the dripper and planting of the highly root active quandong germinant close to the dripper would lead to very early attachment of the parasite to a substantial host. Shade effects are most likely a plant response to water stress in unhosted *Santalum* seedlings. For example, Radomiljac et al (1999b) indicated that unhosted seedlings of *S. album* had higher root to shoot dry weight ratios. Shading is highly beneficial to the transplantation of desiccation prone pot grown quandongs in the field (Lethbridge 1995).

2.4.3 Unbalanced parasite: host growth rates

Whilst the excellent growth demonstrated by quandong when planted near *A. victoriae* is very appealing, care must be taken not to have significant imbalances in the size of host: parasite interactions. A study by Brand et al (2001) with *S. spicatum* and *A. acuminata* shows that if the acacia species is disprortionately small its rate of survival was markedly reduced. The *Acacia victoriae* in this experiment are starting to show significant signs of stress (terminal phyllode loss and yellowing) having to support the largest quandongs in the trial. It is clear from the results that irrespective of the growth rate of the acacia, the quandong will grow unimpeded until the death of the acacia occurs. The current trial was set up under nutrient and water limiting conditions to produce maximum differences in effect in the growth rate of quandongs grown near each acacia species. Care must be taken to have sufficient nutrient and water supply for efficient host growth. Under the current regime the *A.. victoriae*/quandong parasitism will probably not persist. One specimen of *A. victoriae* has already succumbed to the potentially lethal parasitic association. The drought conditions of 2002 at Port Augusta (see appendix 1) probably exacerbates the situation. Planning of host: parasite densities, positioning and species will probably form part of the total management package of the *Santalum* /*Acacia* orchard or plantation (Woodall et al 2002). The other acacia species in the current trial are yet to show obvious stress symptoms.

2.4.4 Suitable growth forms of Acacia for orchard designs

Some general notes on the orchard suitability of the selected acacia species would seem appropriate. Figure 6 shows the average height and width of the selected acacia species. The stand out *Acacia murrayana* will probably require little pruning to make it a suitable orchard tree, an observation also made by Bonney(1997) although some stem splitting was noted. The rounded multistem habit of the other selected provenances of acacia species would need severe pruning to bring them up to standard (single trunk). Other provenance selections may fair better in this respect. After the initial establishment period all species except *A. brumalis* performed well, with only 25 % of *A. brumalis* surviving from the initial planting, some evidence for frost sensitivity was noted. This species natural distribution is from higher rainfall areas of WA and the watering regime used in the experiment may have been insufficient.

Although the juvenile spinescent habit of *Acacia victoriae* is less evident as the plant matures, selection for a completely thornless variety would be well advised. Evidence for sucker development in *Acacia murrayana* or *hakeoides* (Maslin 1998) was not found in this experiment. It should perhaps be pointed out that *Santalum acuminatum* is not adverse to this problem either. To put it in a nut shell, a provenance selection of *Acacia victoriae* that is more upright, thornless with an appropriate nutrition and watering regime would be recommended for growers of quandongs in the Northern pastoral region of SA and areas where *A. victoriae* would be expected to perform well. This would appear to be a highly achievable aim as *A.. victoriae* is a particularly variable and widespread species (Maslin 1998)

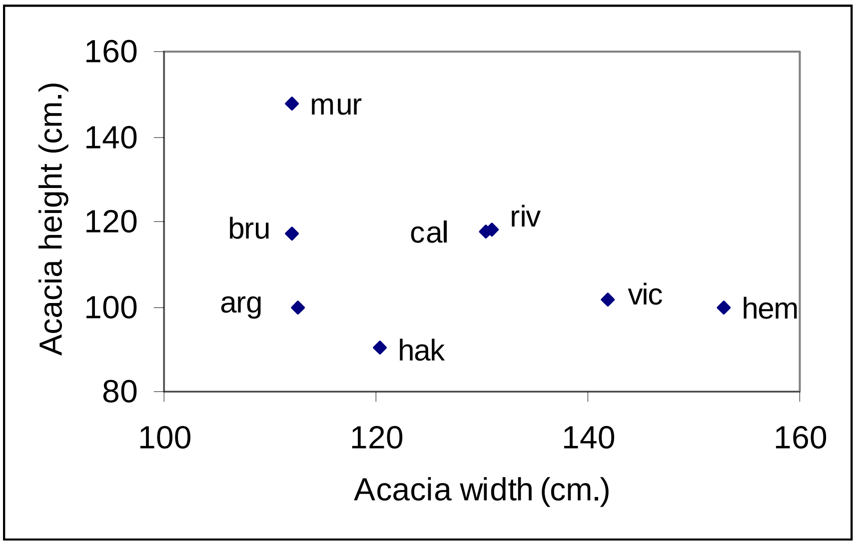


Figure 6. Acacia width (cm.) and Acacia height (cm.) for the indicated species at age 3 yrs (April 2003). See appendix 2 for statistical analysis.

2.5 Appendices

Appendix 1: Meteorological data, Pt Augusta SA

Data from Climate and Consultancy Office of the Bureau of Meteorology, Commonwealth of Australia (latitude 32 degrees 28’ 32” S, longitude 37 degrees 44’ 19” E. Elevation 18.5 m. Most data obtained from the Meteorological site located within the AALBG research area)

	rainfall	(mm.)				
	1998	1999	2000	2001	2002	2003
jan	13.4	19.6	0.8	1	9.8	12
feb	15.6	8.6	31	0	0.8	34
mar	14.2	1.8	20.6	10.4	3	2.4
apr	41.4	6	43.2	7.2	0	
may	2.4	24	10.2	8.2	22	
june	15.4	17.4	6.8	56.6	13.2	
jul	29.8	7.6	17.6	14.2	10.8	
aug	22	20.6	49.4	9.4	3	
sep	17.6	13.2	23.2	58.2	10.6	
oct	24.6	40.8	12.2	30.2	3	
nov	49.4	18	32.6	19.6	13	
dec	-	44	16.4	10	11.2	
	Max	temp	C			
jan	45.4	45.4	43.9	47	43.8	47.8
feb	44.9	41.6	45.2	44	41.8	43.8
mar	41.3	39.4	42.4	38	29.3	37.8
apr	34.5	31.3	31.2	35.1	35.4	
may	27	29.1	25.4	29	31	
june	26.4	22.7	21.8	20	23.2	
jul	19.9	23.9	26	23	26.9	
aug	26.6	29.1	24	26.7	28.1	
sep	37.4	34.1	35	35.9	23.1	
oct	42.6	37.2	37	34.5	36.4	
nov	38.5	34.5	40	35.1	41.5	
dec	-	39.8	43	40.1	45.3	
	Min	Temp	C			
jan	12.9	14.2	12.4	17	12.9	11.7
feb	13.5	13.6	15.5	16	10	15.5
mar	12.6	11.3	8.8	9	10.5	9
apr	5.1	2.8	8.1	5	6	
may	2	3.8	2.7	3	2.5	
june	-1.3	1.2	0.6	0	-4	
jul	-0.3	0.5	0	1.5	-4.1	
aug	2.8	-1.1	2	0.6	-0.8	
sep	4.4	4.3	4	2.8	1	
oct	6.4	6.8	6	4.5	4	
nov	9.9	8.1	13	9.3	8	
dec	-	12.5	13	8.9	10.2	

Appendix 2: Growth data

A species	QH apr2002	Q H oct 2002	Q H apr2003	Q D apr 2003		
arg	52.3	74.7	107.2	14.4		
cal	44.6	66.2	94.3	12.2		
hak	41.2	52.6	72.9	8.2		
hem	50	81	93.3	11.5		
bru	38.3	58.2	75.9	9.6		
vic	72.6	98.2	125.4	19.3		
mur	46.4	68.6	106.2	15.3		
riv	40.1	62	94.3	13.9		
	AH apr2001	s.e.	AW apr2001	s.e.	sig. diff. .05	A %Survival
arg	22.3	2.85	16.17	2.72		55
cal	28.8	3.87	30	4.22		82
hak	48.4	3.55	43.2	3.51		86
hem	62.8	4.61	64.7	5.2		95
bru	64.1	5.62	49.8	6.3		64
vic	38.8	4.34	61	5.81	*	91
mur	65.8	5.02	37.9	3.21	*	91
riv	64.25	5.59	51.1	5.86		91
	AH apr2002	s.e.	AW apr 2002	s.e.		
arg	68.3	12.45	84.5	13.42		
cal	81.1	9.41	116.5	10.9	*	
hak	73.8	5.36	97.7	7.35	*	
hem	77.4	7.19	111.9	11.17	*	
bru	85.8	12.05	86.15	15.92		
vic	80.75	7.53	138	9.66	*	
mur	109.2	12.58	82.75	8.93		
riv	96.5	7.13	114.8	13.3		
	AH Oct2002	s.e.	AW oct2002	s.e.		
arg	89.9	13.01	112.7	16.45		
cal	113.5	7.99	142.4	7.48	*	
hak	86.5	5.65	115.38	7.32	*	
hem	94.9	8.39	160.59	7.19	*	
bru	114.4	18.32	97.86	27.49		
vic	103.8	10.38	159.23	11.33	*	
mur	130.8	16.98	100	11.66		
riv	112.5	10.84	130	19.34		
	AH apr2003	s.e.	AW apr2003	s.e.		
arg	100	13.93	112.73	15.6		
cal	117.5	7.3	130.36	9.48		
hak	90.38	5.35	120.38	6.52	*	
hem	99.69	8.7	152.82	6.72	*	
bru	117	22.89	112	29.56		
vic	101.79	8.86	141.92	9.86	*	
mur	147.8	13.65	112	9.62	*	
riv	118.21	11.16	131.43	16.44		

Key		Key	
A	Acacia	s.e.	standard error
Q	quandong	apr	April
H	height	oct	October
D	diameter	sig. Diff .05	H=W T test (p=0.05)
W	width		

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3. Detecting polyploid quandong in herbarium specimens

By Barbara Randell

3.1 Introduction

In arid areas of Australia, the production of quandong fruit for human consumption is a developing industry. This industry is hampered by several factors in the breeding system of this native tree (Santalum acuminatum (R.Br.) A. DC. - Santalaceae). In particular, plants grown from seed collected from trees with desirable fruit characters do not breed true to the parent tree. And grafted trees derived from a parent with desirable fruit characters are not always self-pollinating. This leads to problems in sourcing orchard trees with reliable characteristics, and also problems in designing orchards to provide pollen sources for grafted trees.

It was suggested that natural polyploid individuals (ie. those with multiple sets of chromosomes in every cell) might be more likely to be self-fertile, and show less variability between seedlings from one tree, than diploids (ie. those with the normal two sets of chromosomes). They might also be easier to interbreed. They might thus solve some of the problems faced in domesticating this native fruit.

Polyploidy occurs in other native woody species (e.g Senna, Randell, 1970, 1989), and it may also occur in quandong. Ideally, it would be associated with a clear morphological marker (such as leaf width, or flower length) which would give growers an easy method to identify and select polyploid individuals in the field.

Previous studies have shown that the size of some plant cells, which are preserved in herbarium specimens, varies directly with the number of chromosomes contained in the cell nucleus. In particular, pollen grains of polyploid plants of Senna are significantly larger than those of their diploid relatives (Randell, 1970). The same relationship holds for the size of stomate guard cells in some other plants (Briggs & Walters, 1984, p. 244).

As both pollen grains and stomates are preserved in dried specimens, the current project surveyed pollen diameters and stomate (guard cell) lengths, in a number of herbarium specimens from many parts of South Australia, to look for indications that more than one ploidy race is present. If analysis showed two distinct peaks in the frequency of the different sizes for pollen grains and/or guard cells, this would be an indication that two different ploidy races were present in the plants sampled.

A number of other characters which might be associated with polyploidy were also measured (eg. leaf width, leaf length, pollen fertility), in the hope that marker characters could be recognised.

3.2 Materials and methods

The fifty plants sampled (table 1, Appendix) bore mature flower buds, and well-developed leaves. These were all the plants in the South Australian State Herbarium, which were collected in South Australia, and had appropriate buds. Some also bore mature fruits or were associated with mature fruits.

The average stomate diameter of a plant was measured as follows. A ‘nail-polish peel impression’ was created by painting the lower epidermis of a single leaf with clear nail polish, allowing the polish to dry, covering firmly with clear adhesive tape, then removing both tape and nail polish together. The tape prevented the polish from stretching as it was removed. When the tape was transferred to a glass microscope slide, the polish carried an impression of the stomates against which it dried. The width of the stomates (guard cells) across the pore could then be measured in the nail polish layer. 10--15 stomates were measured before the average was calculated.

The average pollen diameter for a plant was measured as follows. A single mature bud (the largest unopened bud in an inflorescence containing all flowering stages from immature buds to open, spent flowers) was soaked in detergent and water for several minutes, before the anthers (usually four) were dissected out into water faintly coloured with safranin. The anthers were then squashed to release the pollen grains before the debris of the anthers was removed, and the cover slip applied.

In many cases, the pollen released by a single flower was quite variable. The range included grains which were oval, well-formed, with obvious cytoplasmic contents which absorbed safranin, and with 3 median pores, as described by Sedgley (1982). The exine showed no obvious ornamentation, but its structure was not investigated in detail in this study. Grains showing these characteristics were assumed to be ‘normal’.

By contrast, the same flower often produced grains which were tiny, and/or without cytoplasmic contents, and/or miss-shaped with the walls collapsing inwards. These grains were assumed to be abnormal.

To calculate the average stomate diameter, 10--15 ‘normal’ pollen grains were measured before the average was calculated (ie. empty, miss-shaped, or very small grains were excluded from the calculations).

Pollen fertility was estimated by eye, surveying the entire surface of the cover slip, and comparing the number of ‘normal’ pollen grains with the total number of grains present. Three fundamental assumptions underlie these estimates - ie. that pollen grains which appear ‘normal’ will germinate and function normally; that all flowers on the plant will show the same levels of pollen fertility as a single sampled flower; and that fertility levels displayed in the season the herbarium specimen was collected are typical of those displayed in other seasons, regardless of environmental conditions. None of these assumptions has been tested.

For average leaf widths and leaf lengths, 10--15 measurements of each character were made, before the averages were calculated.

3.3 Results

Table 1 (appendix) includes mean character values for each specimen examined. For each individual character, the recorded range of means was observed, divided into appropriate regular intervals, and the number of records within each interval was scored. (Tables 2 to 6).

Table 2
Average Stomate width

Average Stomate width (μm)	No of records
20-22.49	0
22.5-24.9	1
25-27.49	3
27.5-29.9	8
30-32.49	12
32.5-34.9	11
35-37.49	7
37.5-39.9	7
40-42.49	4
42.5-44.9	0
45-47.5	1

Mean: 32.13
Standard deviation: 8.94

Table 3
Average Pollen Diameter

Average Pollen diameter (μm)	No. of records
16-16.9	1
17-17.9	6
18-18.9	10
19-19.9	14
20-20.9	8
21-21.9	4
22-22.9	4
23-23.9	1

Mean: 18.43
Standard deviation: 4.86

Table 4
Average Leaf length

Average Leaf length (mm)	No of Records
30-39	1
40-49	6
50-59	8
60-69	16
70-79	11
80-89	5
90-99	2
100-110	1

Mean: 61.99
Standard deviation: 20.68

Table 5
Average leaf width

Average leaf width (mm)	No of Records
3-3.9	1
4-4.9	5
5-5.9	10
6-6.9	8
7-7.9	10
8-8.9	8
9-9.9	4
10-10.9	3
11-11.9	0
12-12.9	0
13-13.9	1

Mean: 6.75
Standard deviation: 2.47

Table 6
Estimated Pollen fertility

Estimated pollen fertility	No of Records
0-9	0
10-19	1
20-29	5
30-39	2
40-49	2
50-59	6
60-69	0
70-79	5
80-89	6
90+	15

Mean: 62.6
Standard deviation: 30.22

3.4 Discussion

Tables 2 to 5 display the frequency of records within each interval of mean values of most characters examined (pollen diameter, stomate width, leaf length, and leaf width). Each table shows only a single peak frequency ie. there is no evidence for the presence of two cell sizes, or two leaf sizes, or two ploidy races within the plants sampled.

Table 6 indicates that many plants show significant levels of male (pollen) sterility; ie. that although male gametes are produced, many of them will not function normally. Some plants which show high male-sterility can set fruit (eg. Chinnock 1531, 30% fertile with attached immature fruit). Previous work on one plant (Sedgley, 1982) has shown 50% female-sterility in a sample of 48 flowers (ie. flowers that appear normal, but do not contain female gametes).

Groups of plants which never set fruit (eg. in coastal reserves south of Adelaide, at Hallett Cove Conservation Park, and Aldinga Scrub) may be either female-sterile and/or male-sterile, and/or self-incompatible. Conversely, isolated plants which set good fruit (reported by, for example, D. Matthews pers. com.) must be highly fertile ie. male-fertile, female-fertile and self-compatible (see also Sedgley 1982).

Plants that show high male-sterility sometimes occur in close proximity with plants that show high male-fertility eg F.D.Morgan s.n. (19.12.1983) and D.E.Symon 13607 (19.12.1983) Porter Bay near Pt Lincoln, 90% and 20% fertile; R. Pearce s.n. (23.1.1965), and R. Pearce s.n. (Feb.1965) Midgee Rocks area, 50% and 70% fertile; E.H. Ising s.n. (1938) and H. Amtsberg s.n. (1972) Melrose, 30% and 95% fertile; P.J. Lang 246 (1974) and A.G.Spooner 10628 (1987) Normanville sand dunes 15% and 50% fertile (all cited in Table 1).

3.4.1 Consequences of pollen sterility

For orchard purposes, it is important to select trees for male-fertility as well as fruit characters. The only reliable pollen sources currently available are isolated trees which are known to fruit well. These could be used as the source of pollen-producing grafts, perhaps as single branches in trees selected for fruit characteristics. It is apparent that insects are required to transfer pollen even in flowers of trees that are both male-fertile and female-fertile (Sedgley, 1982).

3.5 Appendix

Table 1– Herbarium specimens sampled

	Locality	Leaf width mm	leaf length mm	pollen diam. um	stomate diam. um	pollen fert %	fruits
1	2 mi inland from clifftops, Koonalda SA (with loose fruits)	5.4	45		28		loose
2	Arkaroola Sanctuary	8.7	76.3	16.4	28.1		
3	c. 1 km south Freeling	7.5	64		31		
4	John Rd Reserve on S boundary of Salisbury East Regional Park	13.3	56.6	18.3	35.8		
5	2km SE of Anna Ck homestead; 16km W of William Ck	10.1	78	18.8	33.5		
6	Coffin Bay Peninsula, near W end of Pt Longnose	9.1	61	19	42.25		
7	10 km south west of Pt Clinton	9.6	85.8	19.5	41	50	
8	ca. 11 km east of Eudunda	7.4	66	20.5	46.5	20	
9	c. 10 km west of Murray Bridge, .5 km west of Railway crossing	4.6	60.8	19.75	34.3	90	
10	Reserve 4 miles east of Two Wells	9.1	83	18.5	32.3	90	
11	Across from Goolwa effluent ponds	8.3	71	19.75	34.8		
12	1 mile west from Mannum	3.5	33	18.3	29.5	90	
13	scrub to north east of Barossa reservoir (with loose fruits)	6.1	51.6	19	26.9	85	
14	Normanville- top of north dune	7.8	73.3	18.5	40.5	50	
15	Grange Golf course	5.2	57.5	20.8	35.5	70	
16	19 km east Oparinna Spring, west end of Musgrave Range	10.4	100.8	17	30.5	40	
17	Sellicks Beach scrub	8.7	64.2	18.1	29.5	90	loose
18	roadside dune vegetation, Point Souttar	8.2	60.7	19.5	36	50	
19	Bower Road, Semaphore	10.3	51	18.25	35.8	85	
20	10km east of Secret Rocks, Cowell-Kimba road	4.5	57.5	21.3	31.8	90	
21	Monarto roadside west from Monarto South	8.5	77	22.3	39.4	90	
22	c. 6 km west of Malinong Hall, c. 45 km SE of Murray Bridge	5.6	42.5	20.4	39.8	90	
23	South Hummocks (34deg.S; 138 deg.E)	4.8	44.2	17.8	32	97	
24	Hundred of Wiltunga, upper Yorke Peninsula	8.8	71	19	33.5	90	
25	Price Cemetary, c. 30 km north of Maitland	5.9	48	22.8	34.8	85	

Table 1. continued

Locality		leaf width mm	leaf length mm	pollen diameter um	stomate diameter um	pollen fert %	fruit
26 West Terrace Cemetary, Adelaide City		4.9	55	18.5	31.25	90	
27 2-3 km north-east of Tanunda township		9	63	23.5	38.25	25	
28 c, 16 km east of Kimba on Main road		6.1	65	20.25	38.5	20	
29 Koonamore, c. 60 km north of Yunta		7.9	78	20.25	37.8	85	
30 Normanville sand dunes, north of Jetty		6.5	60	20.5	34	15	
31 Torrens Road, East Highbury, Adelaide		7.6	63	21.8	37.5	50	
32 Semaphore south		7.4	47	21.3	31.8	85	
33 corner of Robert Rd & Taylors Rd, Angle Vale		6.9	60	17.8	33.3	25	
34 beside road south west of Iron Knob to Kimba		7	70	19.3	31.8	95	
35 Melrose, c. 60 km south east of Pt Augusta		5.5	80	20.3	32.3	30	
36 Melrose Plain, c. 60 km south east Pt Augusta		4.5	60	18.5	24.5	95	
37 Porter Bay, S of Pt Lincoln		8.5	77	20	34.5	20	
38 2.5 mi north of Cowell on the Whyalla Road		5.6	46	22.3	38.3	10	
39 Porter Bay, south of Pt Lincoln		6.1	54	17.5	28.3	90	
40 Australian Arid Lands Botanic Garden, Pt Augusta		8.7	81.6	19.3	42	85	
41 sand dunes near Redcliffe Point	(with attached fruit)	5.2	63.3	17.8	32.8	30	attached
42 Buckleboo, County Buxton		6.5	98	19.8	35.3	95	
43 road to Midgee Rocks from Cowell, Eyre Pen.		5.8	53	19.5	31.8	50	
44 c. 35 km alomg Kingoonya road from Pt Augusta		5.5	68	21.3	31.3	75	
45 Bimbowrie, c. 80 km west of Cockburn	(with loose fruit)	7.6	88	17.3	30.8	70	loose
46 Koonamore Vegetation reserve, Yunta		6.2	69.1	19.8	36.8	70	
47 Canopus Homestead c. 75 km north Renmark		7.6	90.8	22.8	33	55	
48 Ucontitchie Hill (33 deg S,135 deg E) Eyre Pen		6.6	75	18	29.5	90	
49 9km north north east of Yarna Homestead (32 deg S, 135 deg E)		7.5	79.2	19.5	29.5	40	
50 Midgee Rocks area, on road to Mitchellville, NE Cowell		5.4	61.7	19.5	35	70	
means of values		6.74	61.99	18.43	32.13	62.6	
standard deviation		2.47	20.68	4.86	8.94	30.22	

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4. Implications and recommendations

The implications of this research are quite profound. From a quandong production perspective, choosing an inappropriate host could retard the efficiency of a quandong orchard. Sound scientific evidence would suggest that *Acacia victoriae* would make a relatively good host. Simplifying the agronomy of the host-parasite association using water efficient, nutrient efficient species should lead to broader scale planting of quandongs and acacias. A bi-product of this would be the production of wattle seed. Increased quality and quantity of reliable wattle seed production could improve expansion prospects in markets for this product. The high salt tolerance of both *Acacia victoriae* and *Santalum acuminatum* has not gone without notice. With the current national emphasis on salinity remediation, this combination of plants may offer a real alternative to horticulturalists and farmers.

It is unfortunate that every good idea does not bloom, but the lack of evidence for polyploidy in quandong will mean that traditional breeding techniques are probably the best avenue for variety improvement. The use of genetic modification is a possibility with the development of clonal techniques of somatic embryogenesis in other *Santalum* species, but in the native food industry at least, organic production would seem to have taken a stranglehold on both marketing and production.

It is recommended that elegant wattle (*Acacia victoriae*) and quandong (*Santalum acuminatum*) receive equivalent attention in selection of appropriate forms for orchard production of product, using traditional selection and breeding programs.