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DETECTING POLYPLOIDY IN HERBARIUM SPECIMENS OF QUANDONG (*SANTALUM ACUMINATUM* (R.Br.) A.DC.)

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Abstract

Stomate guard cells and pollen grains of 50 herbarium specimens were measured, and the results analysed. There was no evidence of the presence of two size classes of these cell types, and thus no evidence suggesting the presence of two or more ploidy races. High levels of pollen sterility were observed, and the consequences of this sterility in sourcing and managing orchard stock are discussed.

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.

Materials: (see listing of herbarium specimens in Table 1):

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

Methods

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 pollen 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.

Results

Table 1 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).

No.	Locality	Collector & No.	Date	Leaf width mm	Leaf length mm	Pollen diam. μm	Stomate diam. μm	pollen fert%
1	2 mi inland from clifftops, Koonalda SA (with loose fruits)	DE Symon 4584	17.ii.1967	5.4				
2	Arkaroola Sanctuary	RH Kuchel 2997	19.x.1971	8.7	76.3	16.4	28.1	
3	c. 1 km south Freeling	DN Kraehenbuehl 1562	27.xi.1965	7.5	64		31	
4	John Rd Reserve on S boundary of Salisbury East Regional Park	R Taplin 46	20.i.1988	13.3	56.6	18.3	35.8	
5	2km SE of Anna Ck HS; 16km W of William Creek	FJ Badman 1161	3.vi.1984	10.1	78	18.8	33.5	
6	Coffin Bay Peninsula, near W end of Pt Longnose	LD Williams 11776	21.i.1981	9.1	61	19	42.25	
7	10 km SW of Pt Clinton	LD Williams 7560	21.i.1976	9.6	85.8	19.5	41	50
8	ca. 11km E of Eudunda	HM Cooper s.n.	Oct. 1941	7.4	66	20.5	46.5	20
9	c. 10km W of Murray Bridge, 5km W of Railway crossing	JZ Weber 3683	22.i.1974	4.6	60.8	19.75	34.3	90
10	Reserve 4 miles E of Two Wells	JB Cleland s.n.	3.iii.1960	9.1	83	18.5	32.3	90
11	Across from Goolwa effluent ponds	DE Murfet s.n.	1987	8.3	71	19.75	34.8	
12	1 mile west from Mannum	MCR Sharrad 957	1.ii.1961	3.5	33	18.3	29.5	90
13	scrub to north east of Barossa reservoir (with loose fruits)	AG Spooner 6302	4.ii.1979	6.1	51.6	19	26.9	85
14	Normanville - top of north dune	AG Spooner 10628	4.iv.1987	7.8	73.3	18.5	40.5	50
15	Grange Golf course	TJ Smith 1139	15.i.1968	5.2	57.5	20.8	35.5	70
16	19km E Oparinna Spring, W end of Musgrave Range	HH Finlayson	Dec. 1932	10.4	100.8	17	30.5	40
17	Sellicks Beach scrub	JB Cleland s.n.	26.i.1963	8.7	64.2	18.1	29.5	90
18	Roadside dune vegetation, Point Souttar	AG Spooner 6317	2.iii.1979	8.2	60.7	19.5	36	50
19	Bower Road, Semaphore	DN Kraehenbuehl 560	7.iii.1962	10.3	51	18.25	35.8	85
20	10km E of Secret Rocks, Cowell-Kimba Road	DN Kraehenbuehl 4096	5.ii.1984	4.5	57.5	21.3	31.8	90
21	Monarto roadside west from Monarto South	CR Alcock 5508	11.i.1977	8.5	77	22.3	39.4	90
22	c. 6km W of Malinong hall, c. 45km SE of Murray Bridge	MCR Sharrad 1295	30.i.1962	5.6	42.5	20.4	39.8	90
23	South Hummocks (34°S; 138°E)	JS Womersley 37	24.ii.1963	4.8	44.2	17.8	32	97
24	Hundred of Wiltunga, upper Yorke Peninsula	B Copley 1051	5.i.1967	8.8	71	19	33.5	90
25	Price Cemetary, c. 30km north of Maitland	SP Culic for LD Williams 7980	na	5.9	48	22.8	34.8	85

No.	Locality	Collector & No.	Date	Leaf width mm	Leaf length mm	Pollen diam. μm	Stomate diam. μm	pollen fert%
26	West Terrace Cemetary, Adelaide City	DN Kraehenbuehl 4826	13.ii.1987	4.9	55	18.5	31.25	90
27	2–3 km north-east of Tanunda township	DN Kraehenbuehl 2980	20.i.1967	9	63	23.5	38.25	25
28	C. 16km E of Kimba on Main Road	R Hill 1515	17.i.1965	6.1	65	20.25	38.5	20
29	Koonamore, c. 60km north of Yunta	TGB Osborn	Dec. 1924	7.9	78	20.25	37.8	85
30	Normanville sand dunes, north of Jetty	PJ Lang 246	30.xii.1974	6.5	60	20.5	34	15
31	Torrens Road, East Highbury, Adelaide	AG Spooner 729	25.iii.1970	7.6	63	21.8	37.5	50
32	Semaphore South	TJ Smith 1125	8.ii.1968	7.4	47	21.3	31.8	85
33	Corner of Robert Road and Taylors Road, Angle Vale	DN Kraehenbuehl 4718	1.iii.1986	6.9	60	17.8	33.3	25
34	Beside road SW of Iron Knob to Kimba	DD Cunningham 1226	4.i.1996	7	70	19.3	31.8	95
35	Melrose, c. 60 km SE of Port Augusta	EH Ising s.n.	28.xii.1938	5.5	80	20.3	32.3	30
36	Melrose Plain, c. 60km south east Pt Augusta	H Amtsberg s.n.	20.i.1972	4.5	60	18.5	24.5	95
37	Porter Bay, S of Pt Lincoln	DE Symon 13607	19.xii.1983	8.5	77	20	34.5	20
38	2.5 mi north of Cowell on the Whyalla road	R Pearce s.n.	Feb. 1965	5.6	46	22.3	38.3	10
39	Porter Bay, south of Port Lincoln	FD Morgan s.n.	19.xii.1983	6.1	54	17.5	28.3	90
40	Australian Arid Lands Botanic Garden, Pt Augusta	JR Zwar 56	22.xii.1992	8.7	81.6	19.3	42	85
41	Sand dunes near Redcliff Point (with attached fruit)	RJ Chinnock 1531	31.viii.1974	5.2	63.3	17.8	32.8	30
42	Buckleboo, County Buxton	RD Rohrlach 37	17.i.1959	6.5	98	19.8	35.3	95
43	Road to Midgee Rocks from Cowell, Eyre Peninsula	R Pearce s.n.	23.i.1965	5.8	53	19.5	31.8	50
44	C. 35km along Kingoonya Rd from Pt Augusta	TRN Lothian s.n.	4.i.1956	5.5	68	21.3	31.3	75
45	Bimbowrie, c. 80km W of Cockburn (with loose fruit)	W Pearce jnr	1892-95	7.6	88	17.3	30.8	70
46	Koonamore Vegetation reserve, Yunta	B Lay 2	30.i.1970	6.2	69.1	19.8	36.8	70
47	Canopus Homestead, c. 75km north of Renmark	KJ Mack s.n.	9.i.1969	7.6	90.8	22.8	33	55
48	Ucontitchie Hill (33°S, 135°E) Eyre Peninsula	PE Hornsby s.n.	March 1993	6.6	75	18	29.5	90
49	9 km NNE of Yarna Homestead (32°S, 135°E)	LD Williams 9134	15.ii.1977	7.5	79.2	19.5	29.5	40
50	Midgee Rocks area, on road to Mitchelville, NE Cowell	R Pearce s.n.	Feb. 1965	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

Table 1. Herbarium specimens sampled.

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

Table 2. Average stomate width

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

Table 3. Average pollen diameter

Average leaf length	No. of records
30–39	1
40–49	1
50–59	3
60–69	8
70–79	12
80–89	11
90–99	7
100–110	7

Table 4. Average leaf length

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

Table 5. Average leaf width

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 i.e. 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; i.e. that although male gametes are produced, many of them will not function normally. Some plants which show high male-sterility can set fruit (e.g. *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 (i.e. flowers that appear normal, but do not contain female gametes).

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

Table 6. Estimated pollen fertility

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).

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).

Acknowledgments

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