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## Outcrossing in New Zealand wheats measured by occurrence of purple grain

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**Abstract** The inheritance of the purple pericarp character in wheat was determined in two crosses using a derivative of the New Zealand purple cultivar 'Konini'. In both crosses the purple colour was maternally controlled by dominant duplicate genes, with either recessive homozygote epistatic to the effects of the other gene. At the same time the purple character was used as a marker to determine outcrossing levels in 10 New Zealand cultivars, divided into two groups on the basis of the origin of their parents. The outcrossing rates ranged from 0.14 to 3.95% over the 10 cultivars, but there was no difference between the two groups. Problems of pure seed production are discussed in relation to these results.

**Keywords** wheat; cultivars; outcrossing; purple grain; pure seed

### INTRODUCTION

Bread wheat is predominantly self-fertilising, allowing straightforward seed multiplication for the production of cultivar pure seed. However, some outcrossing does occur but is usually minimised by alternating pure seed blocks of wheat and other cereals. Different wheat cultivars vary in their outcrossing rates from effectively zero to over 3%, and in rarer instances to much higher levels (Keydall 1978; Barradas & Romano 1979–80). In New Zealand off-types attributable to outcrossing are identified every year in the nucleus seed produced by Crop Research Division (CRD), Department of Scientific and Industrial Research. Beginning in the 1970s, spring wheats based upon CIMMYT (Mexico) germplasm have been developed for commercial production (McEwan & Griffin 1985) and a higher incidence of off-types in these new

spring wheats was observed compared to the local autumn-sown cultivars (P. Hadfield pers. comm.).

Purple-coloured wheat was developed in the CRD wheat breeding programme during the 1950s with the idea of using this colour to clearly mark feed-type wheat from breadbaking types (Copp 1965). At Cornell University, United States, using a line from Copp's programme, this concept was followed to produce 'Charcoal', a germplasm line to be used as a marker for feed wheat (Jensen 1977). In New Zealand the purple wheat 'Konini' was released in 1981 (Bezar 1980–82), although this has found its niche as a speciality breadbaking wheat for the wholemeal/kibbled-wheat loaf market.

The pigmentation responsible for the purple colour lies within the pericarp layer of the bran coat, which is of maternal origin. McIntosh & Baker (1967) confirmed that purple colour was maternally inherited and controlled by duplicate dominant genes. The purple colour of 'Charcoal' was also found to be controlled by duplicate genes, although their action was incompletely dominant (Gilchrist & Sorrells 1982). Piech & Evans (1979), using another source of the purple grain colour, found it to be controlled by two complementary dominant genes on chromosomes 3A and 7B. Maternal inheritance with dominance for purple colour means that crossed grain from a purple male parent will all be non-purple, but F<sub>2</sub> grain will all be purple. Therefore to measure outcrossing rates, grain from the target plants must be planted and the occurrence of purple plants determined. This pattern of inheritance has also been utilised by Barabas (1973) who proposed purple colour as a marker for distinguishing non-purple hybrid seed from the purple male parent.

The outcrossing rates in 10 New Zealand wheat cultivars were measured using purple grain colour as the marker. Five recent spring wheats derived from CIMMYT germplasm and five locally bred wheats, usually autumn-sown and with no CIMMYT germplasm in their backgrounds, were used in an attempt to identify any differences between the two groups. At the same time, the inheritance of purple colour in a line derived from 'Konini' was followed in two crosses.

**Table 1** The pedigrees of 10 New Zealand wheat cultivars for which outcrossing levels were determined. (Pedigrees with CIMMYT germplasm in their background are printed in bold.)

Group	Cultivar	Pedigree	Year of release
1	Hilgendorf	S1894/7*/Cross 7/Tainui	1961
	Aotea	Hofed 1/Cross 7//Cross 7/ Dreadnought	1957
	Kopara	Arawa/Gabo//Atson/Selkirk/3/ Arawa/Selkirk//Aotea/Hilgendorf	1973
	Takahe	1066.1/7*/Aotea/4/A. Federation/ 5*/Aotea//Red Aotea/3/Aotea	1974
	Arawa	India 9/2*/Tuscan//Dreadnought/ 3/Cross 7//Federation/Kenya 744	1955
	2	Karamu	<b>Lerma Rojo/Norin 10-Brevor// Yakatana 54/Norin 10-Brevor/3*/ Andes</b>
Rongotea		Raven/1966 ISWRN 430	1979
Oroua		1966 ISWRN 395/Skemer	1979
Tiritea		Raven/1966 ISWRN 430	1981
Otane		<b>Tob 's'-Npo//No66-Ira/Bb-Gallo</b>	1984
Purple wheats		Konini	Fortuna/Arawa//Kopara/Purple Hilgendorf
	Line 961	Konini/Oroua	-

## MATERIALS AND METHODS

Ten plots sown with the grain from individual F<sub>1</sub> plants of Cross 852 (PBI 1476 × 961) and seven plots of Cross 903 (436 01 × 961) were grown in 1984 at a low seeding rate. The female parent in both crosses had red grain. Individual plants within each plot were harvested and inspected for grain colour. Plants were classified into three groups for intensity of purple or into a single non-purple category. Heterogeneity chi-squares were calculated for the individual plots and homogeneous data were pooled. Results of chi-square goodness-of-fit tests, corrected for continuity, are reported as the probability of obtaining a chi-square value as large as that observed for the ratio tested.

The wheat cultivars used in the outcrossing experiment are listed in Table 1 and except for 'Otane' are described by Bezar et al. (1980-82). 'Otane' is described by Anon. (1984). The 10 cultivars are divided into five CIMMYT-derived wheats and five wheats with no CIMMYT germplasm in their backgrounds. All of the wheats in Group 1 of Table 1, usually autumn-sown, are facultative types and will produce grain if spring-sown. Line 961 ('Konini' × 'Oroua') is an advanced generation selection of an early maturing purple spring wheat.

On 21 October 1983, 3-row double plots 3.6 m long of 'Konini' and Line 961 were sown with 15 cm between the rows within the plots and 30 cm between the plots. The 10 target cultivars were

handsown in the spaces between the double plots of 'Konini' and Line 961 for the Group 1 and Group 2 cultivars respectively. A randomised block design with two replications was used. A post-emergence herbicide was applied at 200 g/ha dichloroprop, 50 g/ha MCPA, and 6.2 g/ha dicamba (Trident) to control broadleaf weeds, and two applications of 125 g/ha triadimefon (Bayleton) to control rust diseases. At these application rates there was no observed differential effect on seed set amongst the cultivars studied. As a control, five heads on each target cultivar were covered by packets before flowering. The flowering times of all plots were recorded. The grain from the target plants was inspected for grain colour, then sown the following season in single rows with c. 10 cm spacing between plants. A single head per plant was harvested and inspected for grain colour. If outcrossing had occurred, the plant derived from the outcrossed seed will have only purple grain. Percentage outcrossing was calculated from the number of plants with purple grain after examination of between 250 and 350 plants/treatment.

In 1983 there were flowering-time differences within the treatments. Therefore, in 1984 the experiment was repeated but sowing times were staggered in an attempt to synchronise the flowering times of the target cultivars and their purple surrounds. Sowing occurred three times over a period of 14 days beginning on 17 October, but otherwise the same design and procedure as was used the previous year was followed.

**Table 2** Segregation for purple grain colour derived from 'Konini'.

Cross	Generation	No. plants			Ratio	Chi-square probability
		Purple	Non-purple	Total		
852	F <sub>1</sub>	—	10	10	9 : 7	0.21
	F <sub>2</sub>	10	—	10		
	F <sub>3</sub>	249	220	469		
903	F <sub>1</sub>	—	7	7	9 : 7	0.43
	F <sub>2</sub>	7	—	7		
	F <sub>3</sub>	178	153	331		

## RESULTS AND DISCUSSION

The segregation observed in the two crosses with Line 961 is shown in Table 2. In both crosses, the heterogeneity chi-square was not significant and therefore the individual plot data were pooled. The data support a maternal inheritance controlled by duplicate genes. Gilchrist & Sorrells (1982) found the ratios 9 : 7 and 11 : 5 with equal frequency over eight crosses and four environments. In the monosomic series of crosses made by Piech & Evans (1979), all lines except the critical chromosome-deficient Lines 3A and 7B conformed to a 9 : 7 ratio. In one environment in this experiment both crosses fitted a 9 : 7 ratio, which is best explained by dominance of both genes for purple colour with either recessive homozygote epistatic to the effects of the other gene.

In Cross 852, 60% of the purple plants were classified into the lightest category, 9% into the darkest, and 31% intermediate. In Cross 903 the proportions were 54, 11, and 35%. This variation in colour intensity has also been noted by McIntosh & Baker (1967), Piech & Evans (1979), and Gilchrist & Sorrells (1982), and is probably caused by both the interaction of dosage effects for both purple and red grain colour and segregating modifier genes. This experiment provides no evidence for either explanation, but Gilchrist & Sorrells (1982) found one backcross population did support the presence of modifier genes.

As expected purple grain was not found in the target cultivars grown within either the 'Konini' or Line 961 plots in either year, and no purple plants were found in the progeny from the bagged heads in 1983. The analysis of variance for the outcrossing rates, with all effects random, is given in Table 3. The analysis was carried out on angular transformed data since the percentage values were so low. In 1984 missing values were substituted for 'Arawa' since neither replication grew. In Table 3 the cultivar main effect was partitioned into group

**Table 3** Analysis of variance for outcrossing rates in 10 New Zealand wheat cultivars.

Source of variation	df	ms	vr	
Group	1	80.892	2.885	NS
Cultivar within group	8	28.034	3.251	*
Year	1	22.574	2.618	NS
Cultivar × year	8 (1)	11.530	1.337	NS
Residual	19 (1)	8.622		

**Table 4** Percentage outcrossing in 10 New Zealand wheat cultivars.

Group	Cultivar	Year		Mean
		1983	1984	
1	Hilgendorf	0.76	1.96	1.35
	Aotea	0.14	0.14	0.22
	Kopara	0.62	2.44	1.43
	Takahe	0.29	0.68	0.53
	Arawa	0.14	0.21	0.23
2	Karamu	1.13	0.65	0.94
	Rongotea	2.23	3.37	2.84
	Oroua	1.54	0.71	1.15
	Tiritea	0.55	3.95	1.89
	Otane	0.73	0.31	0.56

(see Table 1) and cultivar within group effects. The cultivar within group effect was the only significant effect.

Although the year main effect and the cultivar × year interaction were not significant, the data from the two years are given in Table 4 since some trends are evident. In 1984 'Rongotea', 'Tiritea', 'Kopara', 'Hilgendorf', and 'Arawa' were sown before the other cultivars in an attempt to synchronise flowering. 'Arawa' did not grow, but the outcrossing rates of the other four cultivars were

all markedly higher in 1984 relative to the remaining cultivars. This confirms the field observation that overall flowering coincided better in 1984 than in 1983.

With all effects random, the comparison of the two groups of cultivars was not significant although within each group there were significant differences (Table 3). This indicates that there were differences in outcrossing rates between different cultivars grown in New Zealand, but these are not linked to their origins. The two cultivars with the highest level of outcrossing are in Group 2, but the next two highest cultivars are in Group 1. The two cultivars with the highest outcrossing rates, 'Rongotea' and 'Tiritea', are sister selections (Table 1), and the other two closely related cultivars 'Aotea' and 'Takahe' are also not significantly different from one another. Hadfield (pers. comm.) was correct in observing relatively higher outcrossing rates in 'Rongotea' and 'Tiritea', but he was incorrect in generalising that all CIMMYT-derived cultivars had similarly high levels. There is no obvious reason why some of these wheats should have higher outcrossing rates than others, although it is probably connected with floral morphology and the degree to which the florets gape while their stigmata are receptive. Such differences in floral morphology are important considerations in selecting parental lines for hybrid wheat breeding, although large differences in female receptivity not dependant on flowering time or floral morphology have been noted (Bingham 1986).

The outcrossing rates measured in this experiment are comparable to those reported for most European wheat lines (Keydel 1978; Barradas & Romano 1979-80). The levels reported in all these studies are at the upper limit of what would normally be expected, since the target plants are exposed to artificially high doses of foreign pollen. Pure seed production practice in New Zealand, which follows internationally accepted standards, should be sufficient to keep outcrossing within acceptable levels. However, 'Rongotea' and 'Tiritea' have outcrossing rates at the upper end of the range and particular attention should be given to separating pure seed blocks of these cultivars.

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