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Pleiotropic effects of the chromosome 2D genes *Ppd1*, *Rht8* and *Yr16*

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Pleiotropic effects of the chromosome 2D genes, *Ppd1* for day length insensitivity, *Rht8* for GA responsive height reduction and *Yr16* conferring adult plant resistance to yellow rust, were investigated using homozygous recombinant lines. By growing the experiment in contrasting UK and Yugoslavian environments it was possible to determine genotype-environmental interactions.

Results show *Rht8* acts solely to reduce height by about 8cm. *Ppd1* also reduces height by a similar degree, possibly a consequence of it shortening the growing period by about eight days. In both environments *Ppd1* also reduces spikelet number and increases fertility. Environmental interactions of this gene lead to a production of larger grains and a positive yield increase only in Yugoslavia. The susceptible *yr16* allele acts similarly to *Ppd1* in reducing the number of days to ear emergence and the number of spikelets and in increasing fertility. Effects of this gene are however smaller. Again environmental interactions with grain development influence final yield giving an increase only in Yugoslavia.

Chromosome 2D of bread wheat (*Triticum aestivum* 2n=6x=42) is known to carry several genes of economic importance. These include *Ppd1*, a gene for day length insensitivity (Welsh et al 1973), *Rht8*, an important gibberellic acid responsive dwarfing gene derived from the old Japanese variety Akakomugi and *Yr16*, a gene for adult plant resistance to yellow rust (Worland & Law 1986).

Studies of reciprocal monosomics developed between chromosome 2D of the Yugoslavian variety Sava and the French variety Bersée have demonstrated the importance of the gene array on chromosome 2D to the success of Sava as an early flowering, high yielding, semi dwarf winter wheat (Worland et al 1988).
It is thought that Sava chromosome 2D carries the genes *Ppd1*, *Rht8* and *yr16*. Under Yugoslavian conditions this chromosome controls over 96 per cent of the total variation in respect of days to ear emergence present between Sava and Bersée. Accelerating ear emergence time by about nine days effectively helps Sava avoid the worst effects of summer dessication frequently encountered under Mediterranean conditions. As a consequence of this, grain size and ear yield are increased by 16 and 20 per cent respectively. The same gene array is also responsible for an 18cm reduction in height accounting for 75 per cent of the between varietal variation for this character. The only deleterious effect of the Sava 2D gene array as compared to the Bersée homologue is a reduction in spikelet number, presumably a consequence of the shortened growing period. Measurement of grains per ear however show that increased fertility of the remaining spikelets more than compensates for the reduction in spikelet number, resulting in a net increase in grains per ear.

To determine the economic importance of each of the genes *Ppd1*, *Rht8* and *Yr16*, Worland and Law (1986) developed a series of homozygous recombinant lines between the French variety Cappelle-Desprez and a

Table 1. The number of recombinant lines assigned to each of the eight genotypes between *Ppdl*, *Rht8* and *Yr16* and the equations for estimating additive and interactive effects.

Genotype class				No. of lines
Ppdl,	*Rht8*,	*Yr16*	(X_1)	3
Ppdl,	*Rht8*,	*yr16*	(X_2)	1
Ppdl,	*rht8*,	*Yr16*	(X_3)	3
Ppdl,	*rht8*,	*yr16*	(X_4)	5
ppdl,	*Rht8*,	*Yr16*	(X_5)	4
ppdl,	*Rht8*,	*yr16*	(X_6)	2
ppdl,	*rht8*,	*Yr16*	(X_7)	2
ppdl,	*rht8*,	*yr16*	(X_8)	4

Estimate of additive effect

Ppdl	v	*ppdl*	$\frac{1}{8}(X_1 + X_2 + X_3 + X_4 - X_5 - X_6 - X_7 - X_8)$
Rht8	v	*rht8*	$\frac{1}{8}(X_1 + X_2 - X_3 - X_4 + X_5 + X_6 - X_7 - X_8)$
Yr16	v	*yr16*	$\frac{1}{8}(X_1 - X_2 + X_3 - X_4 + X_5 - X_6 + X_7 - X_8)$

Estimate of interactive effects

Ppdl	v	*Rht8*	$\frac{1}{8}(X_1 + X_2 - X_3 - X_4 - X_5 - X_6 + X_7 + X_8)$
Ppdl	v	*Yr16*	$\frac{1}{8}(X_1 - X_2 + X_3 - X_4 - X_5 + X_6 - X_7 + X_8)$
Rht8	v	*Yr16*	$\frac{1}{8}(X_1 - X_2 - X_3 + X_4 - X_5 + X_6 + X_7 - X_8)$
Ppdl	v	*Rht8* v *Yr16*	$\frac{1}{8}(X_1 - X_2 - X_3 + X_4 - X_5 + X_6 + X_7 - X_8)$

substitution line carrying chromosome 2D of the Italian semi dwarf variety Mara in a Cappelle-Desprez background. Cappelle-Desprez is known to carry *ppdl*, *rht8* and *Yr16* and Mara carries the alternative alleles.

Following the classification of 90 homozygous recombinant lines, a sample of 24 lines was selected to permit orthogonal comparisons between each of the eight genotypic classes involving *Ppdl*, *Rht8* and *Yr16*. Lines were grown in England and Yugoslavia. The results of this experiment, which enables pleiotropic gene effects and genotype environmental interactions to be determined for the two contrasting environments, are the subject of this paper.

METHODS AND MATERIALS

Using techniques described by Law (1966, 1967) homozygous recombinant lines were developed between Cappelle-Desprez and a substitution line of Cappelle-Desprez in which chromosome 2D is replaced by its homologue from Mara. An initial hybrid between these lines produces an F_1 in which only chromosome 2D is heterozygous. Backcrossing to a chromosome 2D monosomic of Cappelle-Desprez and selecting monosomic lines gives rise to a series of recombinant chromosomes in the hemizygous state. Subsequent selfing and selection of disomics from each monosomic produces true breeding recombinant lines. Ninety such lines were produced and classified for *Ppdl*, *Rht8* and *Yr16* by Worland and Law (1986). Twenty-four of these lines representing each of the eight possible genotypic classes were selected for the present investigation. The number of lines in each class varied according to availability and are listed in Table 1.

Lines were grown in drilled 2.4 x 1.0 metre plots sown in autumn 1985 at the Institute of Plant Science Research, Cambridge, England and the Institute of Field and Vegetable Crops, Novi Sad, Yugoslavia. Five replicates were sown in England, three in Yugoslavia. Prior to harvesting in the summer of 1986, ear emergence time was determined, plant height was measured and four ears were removed from each plot for detailed analysis of spikelet number, grains per spikelet, fertility of first and second florets, fertility of central florets and 1,000 grain weight. When ripe the plots were combined to determine plot yield.

An analysis of variance was computed for each character. Estimates of additive and interactive gene effects were determined for each environment using comparisons detailed in Table 1. Estimates of genotype environment interactions were also determined using the comparison:

Environmental effect = 1/2(UK additive effect - YUGO additive effect)

RESULTS AND DISCUSSION

Analysis of variance shows highly significant line effects for all characters studied except UK measurements of ear yield (Table 2).

This demonstrates that all characters are controlled by genes located on chromosome 2D. Apportioning the variation due to *Ppd1*, *Rht8* and *Yr16* shows that, under both UK and Yugoslavian conditions, *Ppd1* exerts significant pleiotropic effects on all recorded characters. Conversely *Rht8* acts solely to reduce height. *Yr16*, or a closely linked gene, has small but significant effects on ear emergence, fertility, grain size and plot yield.

For the characters spikelet number, number of grains per spikelet, fertility of first and second florets, number of grains in central florets of each spikelet and grain size, all the variation is accounted for by the three genes *Ppd1*, *Rht8* and *Yr16*. Analysis of ear emergence, height and yield leaves a small but significant residual effect after removal of variation associated with *Ppd1*, *Rht8* and *Yr16*. This could suggest that additional genes operate in the control of these characters or that variation associated with *Yr16* is indeed a result of linked genes and that the residual effect results from limited recombination.

The estimates of the effects of individual genes are detailed in Table 3.

Ppd1 is seen to accelerate ear emergence time by around eight days in both environments (i.e. 3.98 and 4.07cm deviations from parental mid point). This is presumably a direct effect of the gene conferring day length insensitivity permitting earlier floral initiation as soon as vernalization requirements are satisfied. The susceptible allele *yr16* carried in the same parental gene array as *Ppd1* also accelerates ear emergence time by about half a day. This and all other effects of *Yr16* could be the action of a closely linked gene. Interactions occur between *Ppd1* and *Yr16* in both environments. In this experiment no genotype environment interactions affected ear emergence.

Height is reduced by both *Rht8* and *Ppd1*, which probably reduced height by shortening the growing period. Both genes had similar effects which combined together gave a height reduction of around 17cm. No environmental interactions were recorded.

Spikelet number is reduced primarily by *Ppd1*, again a direct effect of this gene shortening the growing period and time available for primordia initiation. The susceptible allele *yr16* also reduces spikelet number. This reduction is not influenced by the environment.

The three characters, number of grains per spikelet, fertility of first and second florets and grains developing in central florets are all under similar genetic control, being improved primarily by *Ppd1* and secondarily by *yr16*. Both genes having significant effects on fertility in both environments acting independently of each other and of the environment, excepting that the control by *Ppd1* of the number of grains developing in central florets shows an environmental interaction, with a greater fertility increase in Yugoslavia.

Grain size showed a highly significant correlation with *Ppd1* in Yugoslavia acting to

Table 2. Analysis of variance of the Cappelle (Mara 2D) recombinant lines for a range of agronomic characters. Material drilled in England and Yugoslavia 1985.

		Source of variation (MS)								
Character	Environment	Lines	*Ppd1*	Resid.	*Rht8*	Resid.	*Yr16*	Resid.	*Ppd1* v *Rht8* v *Yr16*	Resid.
Ear Emergence (days)	UK	98.0***	2161.9***	4.2***	13.5	101.9***	573.2*	76.5*	315.2***	3.1***
	YUGO	53.5***	1208.7***	0.9*	1.7	55.8***	71.7	52.6***	174.3***	0.6***
Height (cm)	UK	231.1***	2750.5***	16.6***	1818.2**	159.0***	0.14	241.7***	678.9***	32.2***
	YUGO	159.2***	2297.1***	62.1***	875.1***	131.1***	40.1	164.6***	468.8***	23.8***
Spikelet no.	UK	3.8***	60.9***	1.2***	1.5	3.9***	13.0	3.3***	10.7***	0.7
	YUGO	6.6***	137.5***	0.7	1.8	6.9***	14.7	6.3***	20.5***	0.6
No. of grains per spikelet	UK	0.146***	1.997***	0.062	0.017	0.152**	0.613*	0.125**	0.036***	0.051
	YUGO	0.144***	2.355**	0.043**	0.001	0.151***	0.787***	0.115***	0.041***	0.027*
per cent fertility 1,2 florets	UK	95.9***	1478.4***	33.0	3.1	100.1**	329.7	85.2**	229.3**	37.5
	YUGO	43.1***	735.6***	11.6**	4.3	44.9***	157.6*	37.9***	120.2***	9.4*
Grains central florets per spikelet	UK	0.044*	0.418***	0.027	0.608	0.044*	0.178*	0.038*	0.105**	0.017
	YUGO	0.065***	0.987***	0.023**	0.002	0.068***	0.403**	0.049***	1.283***	0.209
1,000 grains (g)	UK	30.9***	113.5*	27.1***	1.7	32.2***	244.4**	22.11***	63.4**	16.7*
	YUGO	31.4***	443.9***	1.8*	0.1	34.4***	9.3	32.43***	87.8***	6.7
Ear yield (g)	UK	0.050	0.062	0.050	0.001	0.052	0.054	0.050	0.031	0.059
	YUGO	0.117***	1.287***	0.063***	0.007	0.122***	0.330	0.107***	0.275**	0.047**
Plot yield (g)	UK	1989.9***	11702.0*	1548.4***	814.4	2043.3***	10676.6*	1595.0***	3412.1*	1372.0***
	YUGO	655.4***	3399.0***	530.6***	410.4	665.5***	6531.1***	388.3***	3015.2***	301.3**

*P = 0.05–0.01; **P = 0.01–0.001; ***P = <0.001.

Table 3. The additive, interactive and environmental effects of genes _Ppd1_, _Rht8_ and _Yr16_ on a range of agronomic characters measured on Cappelle (Mara 2D) recombinants drilled in UK and Yugoslavia

Character	Environment	Additive genotype effects			Significant gene interactions	Interactive environmental effects		
		Ppd1	_Rht8_	_Yr16_		_Ppd1_	_Rht8_	_Yr16_
Ear emergence (days)	UK	−3.98***	+0.11	+0.32	_Ppd1_ v _Yr16_ v _Rht8_*	+0.04	+0.07	+0.01
	YUGO	−4.07***	+0.02	+0.31**	_Ppd1_ v _Yr16_*			
Height(cm)	UK	−4.47***	−3.94***	+0.07	–	+0.40	+0.33	+0.28
	YUGO	−5.26***	−3.28***	−0.49	_Ppd1_ v _Yr16_*			
Spikelet no.	UK	−1.20***	+0.02	+0.22**	_Ppd1_ v _Rh8_*	+0.06	+0.05	+0.01
	YUGO	−1.32***	−0.08	+0.21*	–			
No. of grains per spikelet	UK	+0.125***	+0.031	−0.057**	–	−0.017	−0.003	+0.018
	YUGO	+0.159***	+0.036	−0.092***	–			
Per cent fertility 1,2 florets	UK	+3.36**	+0.14	−1.07	–	+0.20	−0.28	+0.08
	YUGO	+2.96***	+0.69*	−1.22**	–			
Grains central florets per spikelet	UK	+0.058***	+0.028*	−0.036**	–	−0.021*	+0.003	+0.016
	YUGO	+0.100***	+0.022	−0.067**	–			
1,000 grains (g)	UK	−0.62	−0.39	+1.42***	–	−1.71***	−0.22	+0.63**
	YUGO	+2.80***	+0.06	+0.16	_Ppd1_ v _Yr16_***; _Ppd1_ v _Rht8_**			
Ear yield (g)	UK	−0.09*	+0.009	+0.012	–	−0.112***	−0.025	+0.35
	YUGO	+0.13*	+0.034	−0.057*	_Ppd1_ v _Yr16_*; _Ppd1_ v _Rht8_*			
Plot yield (g)	UK	−52.8*	−29.0	−7.0	–	−65.4***	−12.0	+36.0*
	YUGO	+78.0*	−5.0	−79.0**	_ppd1_ v _Yr16_*			

*P = 0.05–0.01; **P = 0.01–0.001; ***P = <0.001

permit grain filling before summer dessiccation. In England, with continued grain filling, effects of *Ppd1* are insignificant. This leads to a strong genotype environment interaction for *Ppd1* which also interacts with *Yr16* and *Rht8* in Yugoslavia. In England alone *Yr16* is associated with larger grains, a consequence of this allele reducing fertility. Effects of *Yr16* show environmental influence.

Both ear and plot yield show strong environmental effects, with *Ppd1* significantly increasing yield in Yugoslavia and reducing yield in England, correlated to the effects of the gene on grain size. In Yugoslavia *yr16* also increases yield through increased fertility, an effect which in England is neutralised by reduced grain size. *Ppd1* and *Yr16* interact in their yield control.

CONCLUSIONS

The study of recombinant lines greatly elucidates the pleiotropic effects of genes on the control of important agronomic characters. This experiment demonstrates that Southern European breeders, in selecting *Ppd1*, *Rht8* and *yr16*, have isolated a very good gene array for their environment. However as this and similar experiments conducted over several years show *Rht8* to be associated with a slight, yet insignificant yield reduction, its retention in the breeding programme is perhaps unnecessary.

In the UK, breeders have rightly rejected *Ppd1*. Selection of *yr16* here could prove beneficial if the associated fertility increase proves exploitable. Similarly, although selection of *Rht8* would, in reducing height, incur a slight yield penalty, introduction of this together with a gene which correlates height promotion with an increase in yield could potentially improve yield levels with no net change in height.

As new marker genes become plentiful with advances in RFLP technology, thus allowing beneficial genes to be tagged and followed through successive generations in varietal development, the full potential of conducting recombinant analysis should be reflected in varietal improvement.

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