Theor Appl Genet (1988) 75:474–479

In vitro expression of genes affecting whole plant phenotype – the effect of Rht/Gai alleles on the callus culture response of wheat (*Triticum aestivum* L. em. Thell)

R. J. Mathias * and E. Atkinson

Department of Cytogenetics, Plant Breeding Institute, Maris Lane, Trumpington, Cambridge CB2 2LQ, UK

Received and accepted August 19, 1987
Communicated by G. Wenzel

Summary. Calli were initiated from immature embryos of 12 lines of hexaploid wheat (*Triticum aestivum* L. em. Thell). The lines were from 3 varieties – April Bearded, Bersee and Maris Huntsman – isogenic for the reduced height/gibberellic acid insensitivity (Rht) genes – Rht1, Rht2 and Rht3 – and the 'tall' (rht) allele. The dwarfing genes had significant effects on the growth and morphogenesis of calli. The genes interacted with the 2,4-D in the medium and the varietal background. Calli of each line were cultured in the presence and absence of 1 mg/l of gibberellic acid (GA), but there was no interaction of the Rht genes with GA in vitro. The effect of the Rht genes is discussed in relation to their effects on cellular hormone metabolism and their involvement in previously described chromosome 4B effects in culture.

Key words: Wheat – Callus – Regeneration – Dwarfing genes – Rht

Introduction

Intervarietal differences in the initiation frequency, growth and regeneration capacity of calli from hexaploid wheat have been described by several authors (Shimada 1978; Sears and Deckard 1982; Lazar et al. 1983; Maddock et al. 1983; Mathias and Simpson 1986). The availability of a large number of aneuploid stocks in wheat provides the opportunity to investigate, in detail, the genetic controls that underlie these intervarietal differences by studying the effect of particular chromosomes, or chromosome fragments, on in vitro performance. Early experiments with ditelosomic lines suggested that, in general, whole arm deletions were deleterious to callus initiation (Shimada and Makino 1975) and growth (Baroncelli et al. 1978). However, recent reports have described significant improvements in the in vitro response of calli as a result of specific chromosome substitutions (Mathias and Fukui 1986; Higgins and Mathias 1987). Mathias and Fukui (1986) suggested that substituting chromosome 4B of Cappelle-Desprez into the nucleus of Chinese Spring might modify cellular hormone metabolism and alter the in vitro response. They also noted that loci for the reduced height (Rht) and grass clump (D3) genes were located on the homoeologous group 4 chromosomes.

The Rht and D3 genes have significant effects on the whole plant phenotype, the most notable being a dramatic reduction in plant height (Gale and Youssefian 1985). In Rht lines, the 'dwarfing' effect is caused by a reduction in the length of the internodes of the peduncle, which is itself a result of a reduction in the average length, and some reduction in the number of cells of the internodes (Nilson et al. 1957; Gale et al. 1986). Several Rht alleles are known: the best characterised are Rht1 and Rht2, which are located on the 4A and 4D chromosomes, respectively, and Rht3, a more potent allele of Rht1 (McVittie et al. 1978). D3 is located on chromosome 4B (Hermes 1963) and may occupy the 'Rht' locus on this chromosome (Gale and Youssefian 1985). In addition to their effect on cell size, Rht1 2 and 3 genes have a pleiotropic effect on hormone metabolism. Tall (Rht null) plants produce fewer tillers and increase in height when treated with gibberellic acid (GA). In contrast, Rht lines are insensitive to applied GA, showing little stem elongation, although they do produce more tillers (Gale and Marshall 1973). This GA-insensitivity may be linked to cellular responses to

* To whom correspondence and reprint requests should be addressed

auxin, as cell extension in excised coleoptiles of rht (tall) wheat lines is inhibited by IAA but is unaffected in Rht 3 lines (Flintham 1981).

In order to test whether or not Rht genes could affect the in vitro performance of wheat calli, or are related to the 4B chromosome effect described by Mathias and Fukui (1986), we cultured three rht varieties and their Rht1, Rht2 and Rht3 isogenic lines (Gale and Youssefian 1985).

Materials and methods

Plant material

The plants used in these experiments were three varieties of *Triticum aestivum* L. em. Thell: April Bearded, Bersee and Maris Huntsman. Within each variety four lines were used, isogenic for Rht1, Rht2, Rht3 and rht (tall). All lines were kindly supplied by Dr. M.D. Gale of the Plant Breeding Institute, Cambridge.

Tissue culture

Plants were grown and tissue cultures initiated and maintained as described by Mathias et al. (1986). Approximately 14 days post-anthesis, immature embryos were removed from freshly harvested and surface sterilised grains. Embryos in which the scutellum was 1 mm long were plated onto initiation medium and subcultured at 4 week intervals onto maintenance and regeneration media. Initiation, maintenance and regeneration media contained 1.0, 0.5 and 0.1 mg/l of 2,4-D, respectively.

Approximately 130 embryos of each line were used in the experiment. One third of the calli from each line were maintained throughout the culture period in the presence of 1 mg/l of GA_3 (Sigma), in addition to the 2,4-D in the medium.

Callus growth on initiation and maintenance media was measured as average callus fresh weight. Morphogenic/regenerative responses were recorded as the number of calli with green spots on initiation, maintenance and regeneration media, the number of calli with roots on regeneration medium and the number of calli with shoots on maintenance and regeneration media.

Statistical analysis

The data was analysed using an analysis of deviance as described by Higgins and Mathias (1987). Deviation among the 12 lines was broken down into variation as a result of 3 main effects (variation between the Rht lines, between GA treatments and between the varieties) and their interactions (Rht × GA, variety × GA, Rht × variety, Rht × GA × variety).

Results

More than 99% of the plated embryos initiated callus; no effect of variety, dwarfing gene or GA was observed on callus initiation.

Callus fresh weight

The mean fresh weight of calli on initiation and maintenance media are depicted in Fig. 1 and the analysis of deviance is presented in Table 1.

Table 1. Analysis of deviance for callus fresh weight increase among 3 wheat varieties isogenic for rht, Rht 1, 2 and 3 alleles on initiation and maintenance media, with and without 1 mg/l of GA

Effect	df	Mean change	
		Initiation	Maintenance
variety	2	3.54×10^{-3}***	0.0407***
Rht	3	5.53×10^{-4}***	0.0109***
GA	1	3.64×10^{-5}	0.0000
Rht × GA	3	1.74×10^{-4}	0.0063
variety × Rht	6	2.75×10^{-4}**	0.0135**
variety × GA	2	1.96×10^{-4}	0.0037
variety × Rht × GA	4	2.19×10^{-4}*	0.0043*

The main effects on initiation and maintenance medium were associated with between variety and between Rht differences. There were also significant variety × Rht and variety × Rht × GA interactions (Table 1). There was no direct effect of GA on callus fresh weight. On initiation medium, the variety effect results from the generally higher fresh weight of Bersee calli when compared with both April Bearded and Huntsman. On maintenance medium, the effect is the result of Huntsman's generally lower callus fresh weight. The other significant main effect was Rht. However, Rht may not be an independent effect as the variety × Rht interaction was significant, suggesting that the Rht effect is dependent on the varietal background.

Shoot primordia formation

The mean percentage of calli forming shoot primordia on initiation, maintenance and regeneration media are presented in Fig. 2 and the analysis of deviance is presented in Table 2.

The major effects on initiation medium were variety and Rht differences and the variety × Rht and variety × Rht × GA interactions. There was no direct effect of GA on primordia formation. On maintenance medium, the only significant effect was the interaction of variety × Rht. Variety, Rht and GA effects were significant on regeneration medium as were variety × Rht and variety × GA interactions.

The variety effect on primordia formation was largely due to the poor performance of Huntsman in comparison to April Bearded and Bersee. The main Rht effect, although significant, again depended on the varietal background, as demonstrated by the significant variety × Rht interaction. The main effect of GA on regeneration medium was to inhibit primordia formation. However, the significant variety × GA interaction demonstrates that the effect was not entirely independent, but dependent on the varietal background.

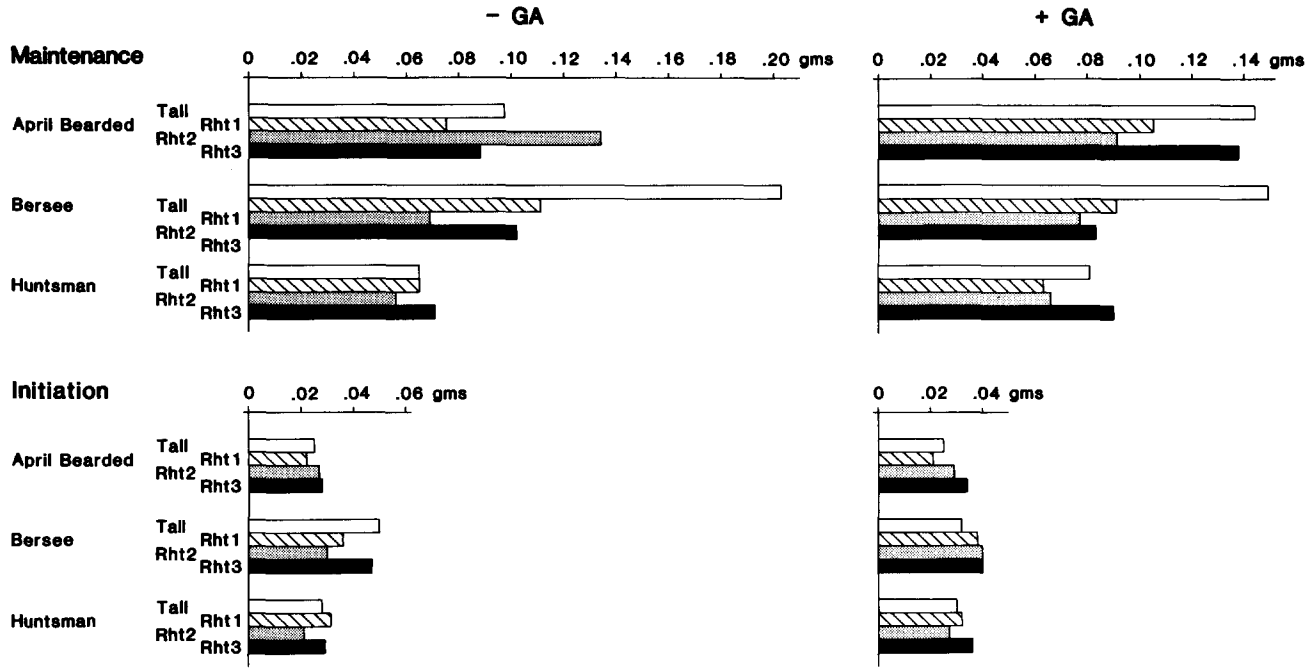

Fig. 1. Average callus fresh weight after 1 month on initiation and maintenance media, with (+*GA*) and without (–*GA*) 1 mg/l of *GA*

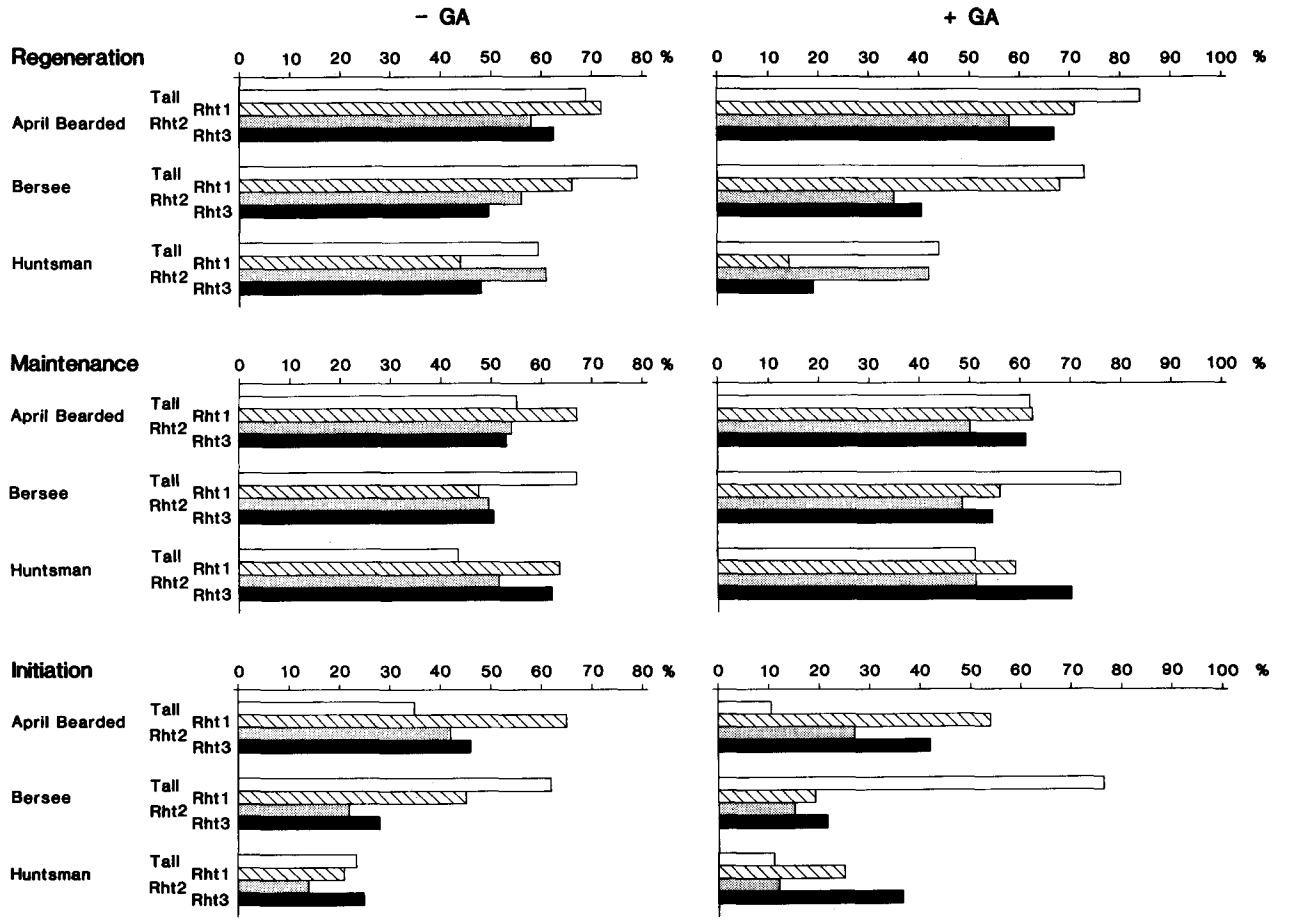

Fig. 2. Percentage of calli with shoot primordia after 1 month on initiation maintenance and regeneration media, with (+*GA*) and without (–*GA*) 1 mg/l of *GA*

Table 2. Analysis of deviance for percentage of calli with shoot primordia among 3 wheat varieties isogenic for rht, Rht 1, 2 and 3 alleles on initiation, maintenance and regeneration media, with and without 1 mg/l of GA

Effect	df	Initiation	Mean change	
			Maintenance	Regeneration
variety	2	39.400***	0.111	26.701***
Rht	3	10.623***	2.171	8.694***
GA	1	1.752	1.869	20.864***
Rht × GA	3	0.829	0.465	1.228
variety × Rht	6	11.726***	4.827***	8.487***
variety × GA	2	1.353	0.210	6.175**
variety × Rht × GA	4	3.860**	0.403	1.869

Table 3. Analysis of deviance for percentage of calli with shoots among 3 wheat varieties isogenic for rht, Rht 1, 2 and 3 alleles on maintenance and regeneration media, with and without 1 mg/l of GA

Effect	df	Mean change	
		Maintenance	Regeneration
variety	2	66.571***	130.678***
Rht	3	6.782***	2.634
GA	1	11.566**	8.370**
Rht × GA	3	1.075	1.944
variety × Rht	6	4.939***	7.902***
variety × GA	2	1.789	1.910
variety × Rht × GA	4	6.153***	3.301*

Root formation

The major effects on root formation were associated with variety and Rht effects and the interaction between them. The main Rht effect was again not independent of the varietal background.

Shoot regeneration

The mean percentages of calli regenerating shoots on maintenance and regeneration media are presented in Fig. 3 and the analysis of deviance for shoot regeneration is presented in Table 3. All of the major effects, except for the Rht effect on regeneration medium, were significant. The variety × Rht and variety × Rht × GA interactions had significant effects on shoot regeneration. The Rht effect on maintenance medium was not independent of varietal background. The significant variety × Rht effect on regeneration medium demonstrates that there was a Rht effect, but that the main effect was not significant. The effect of GA on maintenance medium appears to be to promote regeneration. On regeneration medium GA had the opposite effect, inhibiting regeneration of Bersee and Huntsman but promoting shoot formation in some April Bearded lines. These differences indicate an interaction of GA with 2,4-D in the medium.

Discussion

This is the first report to link a known genetic locus with modified in vitro responses.

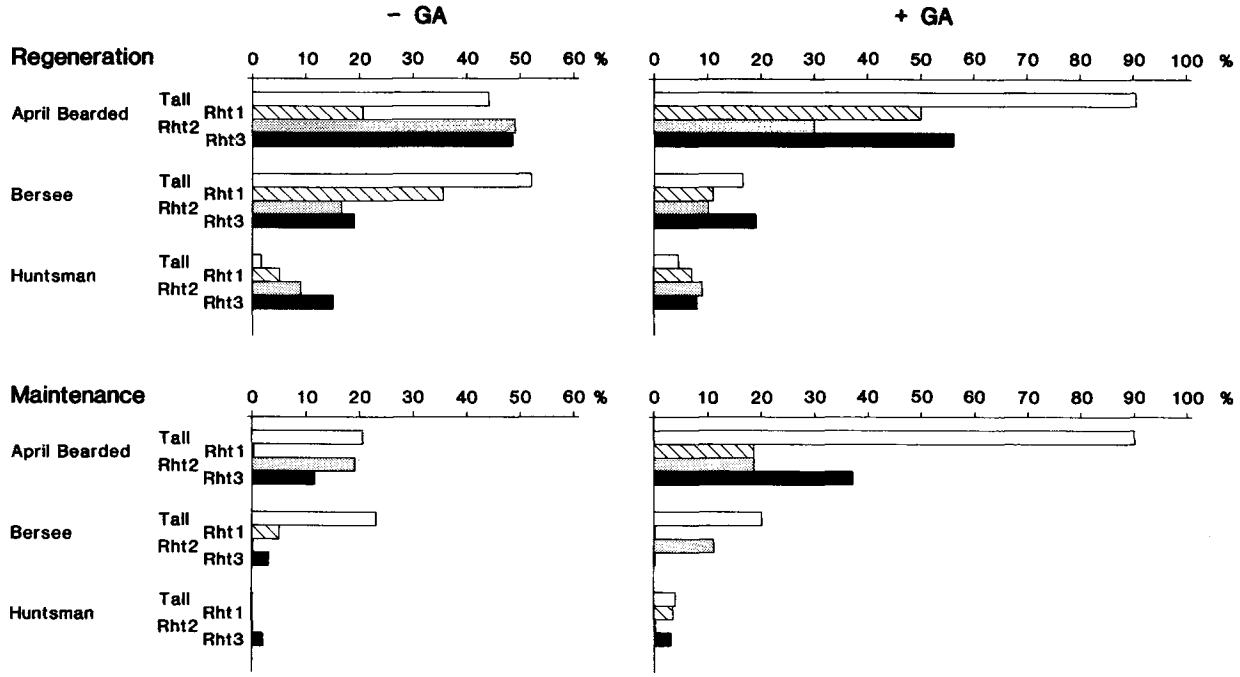

Fig. 3. Percentage of calli with shoots after 1 month on maintenance and regeneration media, with (+*GA*) and without (−*GA*) 1 mg/l of *GA*

Among the main effects (variety, Rht, GA), variety and Rht had significant effects on callus growth and morphogenesis. The interaction of variety with Rht also had significant effects on culture response. Intervarietal differences among wheat calli in culture are well established (Shimada 1978; Sears and Deckard 1982; Lazar et al. 1983; Maddock et al. 1983; Mathias and Simpson 1986). The trend in response for each of the scored characters among the three varieties was:

April Bearded > Bersee > Huntsman.

The significant variety × Rht interactions demonstrate that allelic variation at the Rht locus affects culture response. In vivo the Rht genes result in a fundamental shift in cellular hormone balance (Radley 1970; Flintham 1981; Stoddart 1984). In the seedlings of Rht lines the size of the GA pool is increased (Radley 1970), the accumulation of GA being directly related to the potency of the Rht gene (Lenton et al. 1986). However, although the endogenous GA pool may be 15 times that of tall lines, the rate of GA synthesis is not affected (Stoddart 1984). The Rht alleles are apparently 'active', encoding a product that is responsible for the reduced height phenotype (Rht null = tall), and not responsible for the deletion of an essential step in GA biochemistry (Gale and Marshall 1975). There is some suggestion that the genes operate on a hormone receptor system (Baroncelli et al. 1980; Singh and Paleg 1984).

On different concentrations of 2,4-D the pattern of response among isogenic lines was different, suggesting an interaction of Rht × 2,4-D or Rht × variety × 2,4-D. Some evidence suggests that auxin responses are modified by the Rht genes. First, IAA inhibits the expansion of coleoptiles from tall varieties in vitro, but has no effect on the coleoptiles of Rht3 lines (Flintham 1981). Secondly, apical dominance phenomena, usually associated with auxin effects (Phillips 1969), are modified in dwarf wheats. The tillering of Rht lines is increased by GA application while tall varieties produce fewer tillers (Gale and Marshall 1973). Thirdly, there is a rapid increase in extractable IAA when GA is applied to tall varieties which does not occur in GA insensitive Rht lines (Romanova and Prilyuk 1975). The apparent interaction of the Rht genes with 2,4-D in the medium supports the suggestion that the genes have an effect on both auxin and GA responses.

Although the effects of the Rht/Gai genes in vivo are largely independent of varietal background, no independent Rht effect was found in culture. This was not entirely unexpected, as various in vitro responses are the result of complex interactions between genetic, physiological and exogenous factors; within this complex the Rht genes potentially affect cellular responses to both GA and auxin.

GA has been reported to have no effect on shoot regeneration from wheat calli but to promote root formation (Ozias-Akins and Vasil 1982). In these experiments GA did not affect root formation or callus fresh weight, but there was a GA effect on shoot regeneration on maintenance medium and shoot primordia and shoot formation on regeneration medium. In contrast to the situation in vivo, no Rht × GA interaction was found in vitro. However, as exogenous GA has no dramatic effects on wheat callus cultures, any interaction effects would be difficult to detect.

Under the described culture conditions the dominant media effect appears to be the concentration of 2,4-D in the medium. It is reasonable to assume that the differences in vitro between Rht lines might reflect differences in their sensitivity to exogenous hormones. Mathias and Fukui (1986) suggested that substituting the 4B chromosome of Cappelle-Desprez into Chinese Spring might result in a shift in cellular hormone metabolism, which would alter the sensitivity of cells to exogenous growth regulators. Higgins and Mathias (1987) have since described the interaction of two genes on the regeneration of wheat calli, which they suggest may have their effect via modification of hormone metabolism, stress tolerance or carbohydrate synthesis.

The Rht genes affect cellular and whole plant responses to exogenous auxin and GA, possibly through alteration of receptor systems. The genes also have significant effects in culture through their interaction with the genetic background and hormones in the medium. The demonstration of an Rht effect in culture indirectly supports the suggestion (Mathias and Fukui 1986) that homeologous genes on chromosome 4B might be involved in the effects reported by Mathias and Fukui (1986) and Higgins and Mathias (1987). However, only gene mapping studies will resolve whether the Rht genes and related alleles are indeed involved in the 4B chromosome effects on callus regeneration response.

Acknowledgements. E. Atkinson was supported by EEC Contract GB 1.4.115 UK (H).

References

Baroncelli S, Buiatti M, Bennici A, Froughi-Wehr G, Mix G, Gaul H, Tagliasacchi AM, Loiero M, Giorgi B (1978) Genetic control of in vitro and in vivo growth of hexaploid wheat. I. Behaviour of ditelocentric lines. Z Pflanzenzücht 80:109–116

Baroncelli S, Buiatti M, Magnani G (1980) Control of gibberellin action by "semi-dwarf" genes in durum wheat. Z Pflanzenzücht 84:219–225

Flintham JE (1981) The physiological role and plant breeding potential of the Tom Thumb dwarfing gene in wheat. PhD thesis, University of Cambridge

Gale MD, Marshall GA (1973) Insensitivity to gibberellin in dwarf wheats. Ann Bot 37:729–735

Gale MD, Marshall GA (1975) The nature and genetic control of gibberellin insensitivity and coleoptile length in 'dwarf' wheat. Heredity 34:393–399

Gale MD, Youssefian S (1985) Dwarfing genes in wheat. In: Russell GE (ed) Progress in plant breeding, vol 1. Butterworths, London, pp 1–35

Gale MD, Hoogendoorn J, Salter AM (1986) The effects of dwarfing genes of wheat on cell size and cell numbers. Annu Rep 1985, Plant Breeding Institute, Cambridge, p 69

Hermsen JGTh (1963) The localisation of two genes for dwarfing in the wheat variety Timstein by means of substitution lines. Euphytica 12:126–129

Higgins P, Mathias RJ (1987) The effect of the 4B chromosomes of wheat on the growth and regeneration of callus cultures. Theor Appl Genet 74:439–444

Lazar MD, Collins GB, Vian WE (1983) Genetic and environmental effects on the growth and differentiation of wheat somatic cell cultures. J Hered 74:353–357

Lenton JR, Hedden P, Gale MD (1986) Gibberellin insensitivity and depletion in wheat – cosequences for development. In: Hoad GV, Lenton JR, Jackson MB, Atkins RK (eds) Hormone action in plant development – a critical appraisal. Butterworths, London, pp 145–160

McVittie JA, Gale MD, Marshall GA, Wescott B (1978) The intra-chromosomal mapping of the Norin 10 and Tom Thumb dwarfing genes. Heredity 40:67–70

Maddock SE, Lancaster VA, Risiott R, Franklin J (1983) Plant regeneration from cultured immature embryos and inflorescences of 25 cultivars of wheat (*Triticum aestivum*). J Exp Bot 34:915–926

Mathias RJ, Fukui K (1986) The effect of specific chromosome and cytoplasmic substitutions on the tissue culture response of wheat (*Triticum aestivum*) callus. Theor Appl Genet 71:797–800

Mathias RJ, Simpson ES (1987) The interaction of genotype and culture medium on the tissue culture responses of wheat (*Triticum aestivum* L) callus. Plant Cell Tissue Organ Cult 7:31–37

Mathias RJ, Fukui K, Law CN (1986) Cytoplasmic effects on the tissue culture response of wheat (*Triticum aestivum*) callus. Theor Appl Genet 72:70–75

Nilson EB, Johnson VA, Gardner CO (1957) Parenchyma and epidermal cell length in relation to plant height and culm internode length in winter wheat. Bot Gaz 119:38–43

Ozias-Akins P, Vasil IK (1982) Plant regeneration from cultured immature embryos and inflorescences of *Triticum aestivum* L. (wheat): evidence for somatic embryogenesis. Protoplasma 110:95–105

Phillips IDJ (1969) Apical dominance. In: Wilkins MB (ed) Physiology of plant growth and development. McGraw-Hill, New York, pp 165–202

Radley ME (1970) Comparison of endogenous gibberellins and response to applied gibberellins of some dwarf and tall wheat cultivars. Planta 92:292–300

Romanova LV, Prilyuk LV (1975) Hormonal composition and reaction of dwarf wheats of different origin to gibberellin. Skh Biol 10:750–755

Sears RG, Deckard EL (1982) Tissue culture variability in wheat: callus induction and plant regeneration. Crop Sci 22:546–550

Shimada T (1978) Plant regeneration from the callus induced from wheat embryo. Jpn J Genet 53:371–374

Shimada T, Makimo T (1975) In vitro culture of wheat. III. Anther culture of the A genome aneuploids in common wheat. Theor Appl Genet 46:407–410

Singh SP, Paleg LG (1984) The low temperature induction of hormonal sensitivity in genotypically gibberellic acid-insensitive aleurone tissue. Plant Physiol 74:437–458

Stoddart JL (1984) Growth and gibberellin A, metabolism in normal and gibberellin-insensitive (Rht3) wheat (*Triticum aestivum* L) seedlings. Planta 161:432–438