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Preliminary assessment of the effect of brassinosteroids on yield in winter wheat

by

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1. ABSTRACT

Brassinosteroids (BRs) are an important group of plant hormones essential for growth and development. Released in response to stress, they regulate the expression of numerous genes, including ones associated with plant height and yield. Spraying brassinosteroid preparations has been shown to increase establishment and yield in a number of crops.

The aim of this pilot project was to investigate whether the application of brassinosteroids increases yield in wheat grown under UK field conditions. Spraying the hormone is expensive; a seed treatment could be more cost-effective and so was also tested.

The key messages from these pilot field trials were:

- Spraying wheat varieties grown under UK conditions with a non-commercial formulation of brassinosteroid had no significant effect on yield in unstressed plants.
- However, yield loss in wheat under stress may be lower in wheat sprayed with brassinosteroid.
- Further work under controlled conditions is required to demonstrate the interaction between brassinosteroid compounds and the growth of UK wheat varieties.

2. SUMMARY

The 'green revolution' in wheat and rice arose from the introduction of semi-dwarf varieties. Although dwarfing genes in rice and wheat have different functions, both produce products linked with malfunction of the plant hormone, gibberellin. The reduction in height in wheat increases lodging resistance and harvest index, and is also associated with an increase both in ear fertility and seed size. Given the yield gains achieved by the introduction of gibberellin based semi-dwarfing genes it should be worthwhile examining alternative pathways that influence plant height for future crop development.

Brassinosteroids (BRs) are polyhydroxylated plant sterol derivatives, identified in 1970 from Brassica pollen by USDA scientists. They are ubiquitous in the plant kingdom and are a key plant hormone essential for plant growth and development. BRs are released in response to root, pest, disease or environmental stress but are also known to regulate the expression of numerous genes, including ones associated with plant height and important yield components in crops. Over 60 kinds of BR have been found to date, of which brassinolide, 24-epibrassinolide and 28-homobrassinolide are the three most potent.

One of the classic phenotypes of BR mutation is dwarf or semi-dwarf plants. More than 70% of barley cultivated in Japan is occupied by high yielding semi-dwarf, lodging resistant barley varieties that carry a mutation in a BR gene. Endogenous BR levels have also been associated with height reduction in model species. In addition, exogenous BR application has been shown to increase crop establishment and yield in a number of crops, including wheat.

This project was conceived as a low cost, preliminary investigation to develop a greater understanding of the role of brassinosteroids in regulating plant growth and development in wheat to facilitate assessment of the potential value as an alternative source of genetic variation for yield manipulation in wheat breeding. Specifically this project aimed to test whether spraying wheat with BR or applying it as a seed treatment will increase yield of UK varieties.

The project consisted of a pilot experiment run at two locations; NIAB HQ in Cambridge and Nickerson (UK) Ltd. trial site at Woolpit in Suffolk, using the varieties Einstein, Robigus and Paragon. All three varieties are sensitive to BR application as assessed by a root inhibition assay in the presence of 0.1µg/µl and 1.0µg/µl brassinolide and they cover a range of dwarfing gene (*Rht*) types. Einstein is a winter wheat carrying the *Rht2* semi-dwarf allele, Robigus is a winter wheat with *Rht1* and Paragon is a tall (*rht*) spring variety. This parameter was considered as there may be interaction between the brassinosteroid and gibberellin pathways.

A split plot design was used to optimise the resources used, in terms of treatments and replication. As a pilot scheme covering many potentially important factors, the experimental strategy was likely to detect only “large differences” in varietal response. Five applications of BR at dosages of 0.01ppm of epi-brassinolide (EBL) were sprayed at different stages of crop development. A sixth cumulative five-spray treatment was used to test whether there is any additive effect of spraying with EBL at different stages on the same plants. The trial included an untreated control.

A seed treatment trial was also conducted at two sites in Suffolk. Trials were treated with a full fungicide programme in order to exclude disease. No growth regulators were applied.

A number of standard observations were recorded across the trial. Analysis revealed no significant differences in most observations. However, at one trial site there were significant differences in yield results for Paragon and Robigus where lodging and yellow rust had affected yields. The average yield across the Paragon plots was particularly low, but some BR treatments, particularly the cumulative treatment significantly boosted yield compared to the control. One seed treatment trial site was abandoned due to inclement weather and no significant interactions with BR seed treatment were noted at the second site.

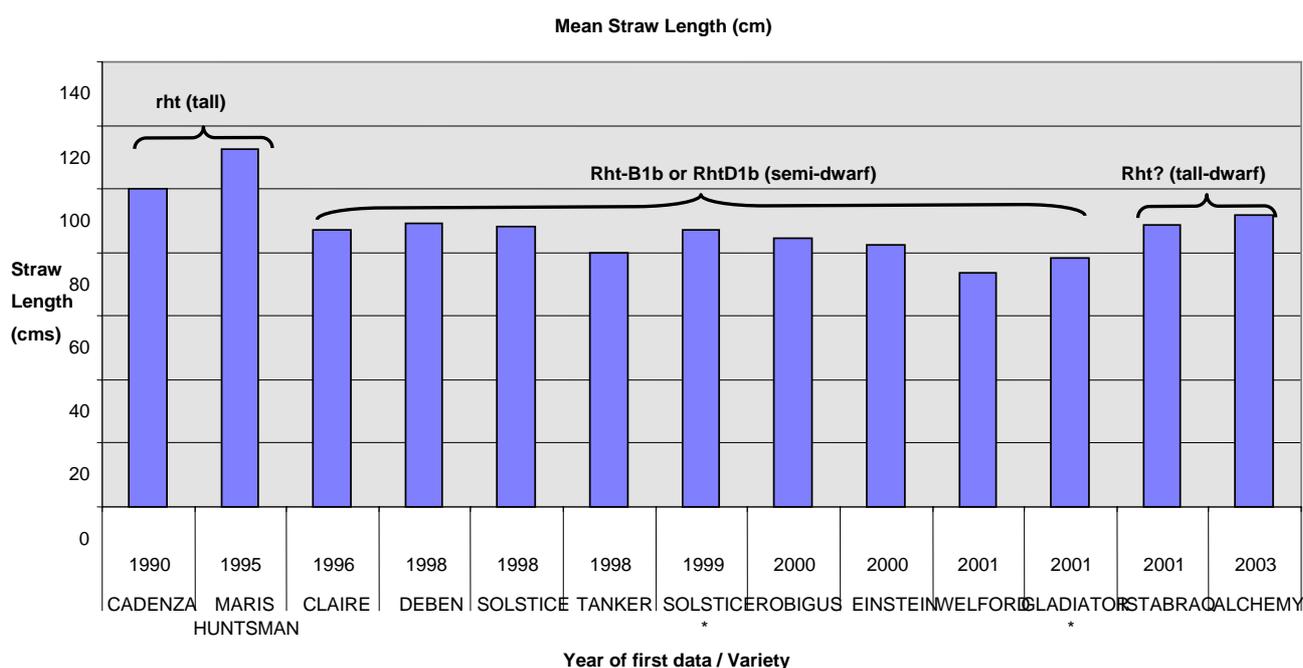
3 TECHNICAL DETAIL

3.1 Introduction

A universal objective of wheat breeding programmes is to maximise yield. Historically, conventional breeding activity in the UK has achieved an increase in yield gain of about 1% per annum. Aside from steady advances, there have been a number of quantum leaps in yield gain, the most spectacular of which was achieved with the introduction of the gibberellic acid (GA) insensitive, semi-dwarfing genes in the 1970s which spurred the ‘green revolution’. Insensitivity to gibberellin is conferred by *Rht* and restricts stem growth. This reduces the susceptibility to lodging and thereby increases harvest index. Parallel to this effect, more energy is diverted into the developing ear and so *Rht* is also associated with an increase both in ear fertility and seed size (Austin *et al.*, 1980). There does, however, remain a general correlation between reduced height and reduced yield (Law *et al.*, 1973).

The majority of the UK wheat acreage is sown to semi-dwarf varieties carrying *Rht-B1b* (formerly *Rht1*) or *Rht-D1b* (formerly *Rht2*). A trend may be developing towards somewhat taller varieties (W. J. Angus, personal communication; Figure 1). Although *Rht* genotypes are always shorter than their *rht* equivalents, penetrance depends on genetic background; thus dwarf varieties carrying *Rht-B1b* or *Rht-D1b* can vary significantly in plant height. Research in the 1970s concluded that amongst *Rht* carriers, relative tallness was associated with higher yield potential as a result of their relatively larger photosynthetic capacity (Gale and Law, 1976). This led to the proposition of the ‘tall dwarf’ ideotype. However, the genetic determinants of this variation in height remain obscure.

Figure 1: Changes in the height of UK wheat varieties 1990 – 2003.



The gains made in UK varieties from the exploitation of *Rht* genes to date are clear, but new height reducing genes that break the correlation between reduced height and reduced yield that could offer improvements on current varieties remain a useful target. At least 21 *Rht* genes have been identified which reduce plant height (McIntosh *et al.*, 1998), but there are often complex interactions with environmental factors which reduces the number of potential use in commercial breeding programmes in Europe (Worland and Snape, 2001). There are often also often undesirable ‘side-effects’ that influence the usefulness of these genes. For example, although *Rht-B1b* and *Rht-D1b* are widely used in UK varieties, their presence is correlated with shorter coleoptiles and reduced seedling vigour (Rebetzke *et al.*, 2001). Temperatures over 24°C during the period 10 days prior to flowering have deleterious effects on yield in *Rht-B1b* and *Rht-D1b* carriers, leaving these varieties unsuited to Southern European climates.

The disadvantageous characteristics of *Rht-B1b* and *Rht-D1b* are not shared by *Rht8* so this gene could be useful. However, the commercial use of *Rht8* has, to date, been confined to the Mediterranean environment of southern Europe (Worland *et al.* 2001), and there is less enthusiasm for a similar switch in the UK, because there is evidence that *Rht8* is unsuitable for the cooler conditions prevalent in northern Europe. Nothing is known about the mechanism of *Rht8*-mediated dwarfism, except that it is gibberellin sensitive (Botwright *et al.* 2005). As other *Rht* dwarfing genes are associated with yield penalties there is a potential role for complementary dwarfing genes for cereals grown in the UK, in particular for genes that also confer good climatic adaptability.

The gibberellin pathway, which is the target of the *Rht* genes, is conserved across the plant kingdom but it is also worthwhile to study other pathways that influence plant height. The brassinosteroid (BR) pathway is attractive not just because endogenous BR levels have been associated with height reduction in model species, but also because exogenous BR application has been shown to increase yield in a number of crops (Mitchell *et al.*, 1970; Ikekawa and Zhou, 1991).

Brassinosteroids (BRs) are polyhydroxylated plant sterol derivatives with structural similarity to growth-regulating steroid hormones found in vertebrates and insects. Although BRs were discovered over 30 years ago (Mitchell *et al.*, 1970), the first report of their effects on *Arabidopsis* growth did not appear until 1991 (Clouse and Zurek, 1991). Since then, the BR biosynthesis and signal transduction pathways have been well studied in *Arabidopsis* and in pea, rice, tomato and barley (Bishop, 2003). The signalling mechanism for the BR response is very similar to that used by gibberellins. In *Arabidopsis*, it consists of receptors encoded by the genes; BR insensitive1 (*BRI1*), BR receptor like (*BRL*) and *BRI1*-associated receptor kinase1 (*BAK1*), which work together to perceive the presence of BR. After perception, a downstream signalling cascade leads to the BR response, which typically involves cell elongation. Mutation in any of these three genes leads to a lack of response to BR,

resulting in no enhancement of height upon BR application. BR mutants fall into two types: BR sensitive (Chory *et al.*, 1991; Li *et al.* 1996; Szekeres *et al.*, 1996; Choe *et al.*, 1999; 2000) and BR insensitive (Clouse *et al.* 1996; Li and Chory, 1997; Li *et al.*, 2001a; b; Zhou *et al.*, 2004). The former include lesions in BR biosynthetic genes, while the latter include lesions in BR receptors and are distinguished by their inability to be rescued by exogenous BR application. The connection between plant height and the BR pathway has been recently demonstrated in barley, where a high yielding, lodging resistant Japanese semi-dwarf variety carrying the *uzu* allele has been shown to harbour a point mutation in a gene homologous to the *BR11* gene of Arabidopsis (Chono *et al.*, 2003). Over 70% of Japanese barley acreage is currently dedicated to *uzu*-carrying varieties. This demonstration that a semi-dwarf phenotype important to cereal productivity can be generated by a mutation in the BR pathway genes is particularly relevant.

Exogenous application of BR has been suggested as a yield-enhancing measure for a number of crop species. Its effects include significant increases in seed yield per plant in bean and rice; in leaf weight in lettuce (Meudt *et al.*, 1983); in stem diameter (and thereby lodging resistance) in barley, in the number of seeds per panicle, in ear weight and in 1000 grain weight in wheat. Similar yield increases have been observed in a number of other crops (Zullo and Adam, 2002). Increasing endogenous BR levels by a transgenic route has also been found to increase yield. Thus in Arabidopsis and tobacco, over-expression of *DWARF4*, which is involved in BR biosynthesis, promoted increased vegetative growth and yield (Choe *et al.*, 2001). Similar results have been obtained in tomato and rice (Flavell, 2005). The exogenous application of BR at low concentrations has been reported to increase coleoptile length and promote root elongation in Arabidopsis and a number of crops (Mussig *et al.*, 2003, Ikekawa and Zhou, 1991). Six-year field trials in China and Japan have reported that spraying wheat with an optimal dose of 0.01 ppm BR (Epi-brassinolide) results in 10-20% yield increase (Ikekawa and Zhao, 1991). Commercial products are available that make yield enhancing claims (for example see: http://www.mikonik.com/default.asp?issue_id=10).

The focus of the present study was to conduct preliminary studies to explore the broad impact of brassinosteroids on wheat crop productivity to underpin the provision of evidence in support of the hypothesis that BR genes may serve as a complementary source of dwarfing genes in the future development of new high yielding UK wheat varieties. The pilot project sought specifically to investigate whether the application of brassinosteroids increases yield in wheat.

Spraying with the hormone is a potentially expensive approach, a seed treatment would be more cost-effective and the pilot project included a trial designed to measure the impact of a brassinosteroid seed treatment on yield. The demonstration of a yield response to this hormone under UK field conditions

either by direct spraying or via a seed treatment would provide preliminary evidence of the potential gains in yield that might be possible from an interaction with brassinosteroid compounds.

3.2 Materials and Methods

3.2.1 Spray Trials

The pilot spray trial experiment was run at two locations; NIAB, Cambridge and Nickerson (UK) Ltd. trial site at Woolpit in Suffolk, using the varieties Einstein, Robigus and Paragon. The varieties were selected to provide a range of Rht status, end-use and breeders. Einstein is a group 2 hard milling winter wheat bred by Nickerson (UK) Ltd that carries the *Rht-D1b* semi-dwarf allele. Robigus, bred by CPB Twyford, is a group 3 soft biscuit winter wheat with *Rht-B1b*. Paragon is a group 1 hard milling spring wheat with the tall (*rht*) allele and is owned by RAGT. All three varieties are sensitive to BR application as assessed by the root inhibition assay in the presence of 0.1ug/μl and 1.0ug/μl brassinolide (BL) (Manash Chatterjee, pers. comm.).

A split plot trial design was used to optimise resources in terms of treatments (see below), and replication. As a pilot scheme covering many potentially important factors, the experimental design was likely to facilitate detection of “large differences” in varietal response only.

A concentration of 0.01ppm epi-brassinolide (EBL) (90% purity) was applied in conjunction with a standard wetting agent. This concentration of EBL had been reported as optimum in previous studies (Ikekawa & Zhao (1991)). The spray trial treatment plan delivered seven treatments in total as follows:

T1	Control	
T2	Stem elongation, Zadoks scale - 30	early April 2006
T3	Booting, Zadoks scale - 40	late May 2006
T4	Heading, Zadoks scale - 50	early June 2006
T5	Flowering, Zadoks scale - 60	mid June 2006
T6	Early dough stages, Zadoks scale - 83	late July/early Aug 2006
T7	Cumulative treatment (T2+T3+T4+T5+T6)	

Trials were treated with a full fungicide programme in order to exclude all diseases. No growth regulators were applied.

Measurements were taken for plant height to tip of ear, ear emergence (growth stage 61 (Zadoks scale)), clean ripening, lodging (%), 1000 grain weight, yield, specific weight, moisture content and

straw biomass. Samples were also prepared for harvest index and grains/spikelet but actual measurement of these parameters was conditional on a positive effect on yield.

3.2.2 Seed Treatment trials

Two pilot seed treatment trials were run at Clopton and Risby in Suffolk. Two seed dressing treatments were used; an untreated control (SCD₁) and seed coated with 0.01ppm epi-brassinolide (90% purity) in conjunction with a standard seed dressing agent (SCD₂). Measurements to be recorded were yield, specific weight and moisture at harvest.

3.3 Results

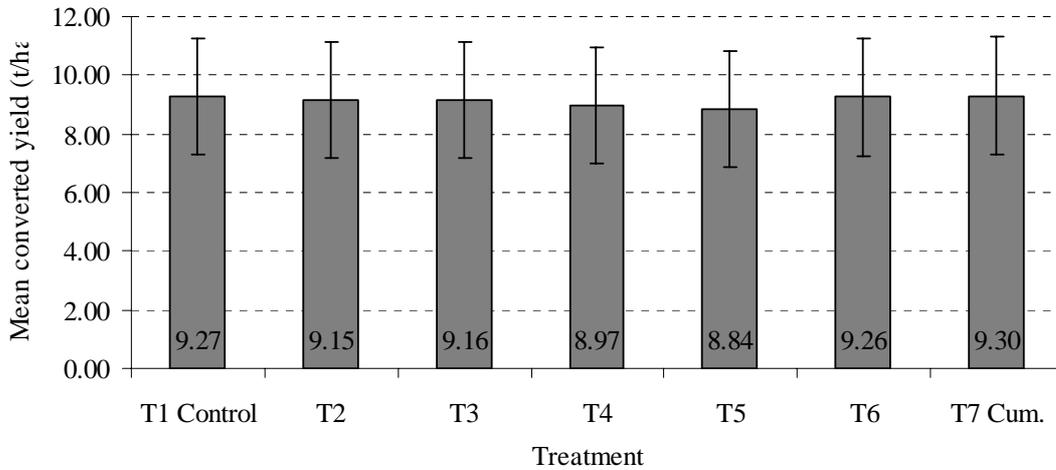
3.3.1 Spray trials

The least significant difference of the means (LSD) for the Cambridge spray trial are reported in Table 1. Overall analysis of the trial results noted significant differences in yield for treatment (at 0.1%), variety (at 0.1%) and the variety, treatment interaction (at 0.5%). No significant differences were detected for the other parameters measured (Table 1).

Further analysis of variance in the yield data contrasting the control and treatments over the whole trial was carried out and detected differences significant at 0.1% between the control and the cumulative treatment (T7) and nearly significant differences at the 5% level for T5 (flowering) and T6 (early dough) (data not presented). Further comparisons for the whole trial between each treatment and the cumulative treatment (T7) highlighted significant differences at 0.1% for stem elongation (T2) and at 1% for booting (T3), heading (T4) and flowering (T5).

Figures 2-4 describe the differences in yield across treatments for each variety. Yields from Einstein were reasonable, but were not significantly affected by the application of EBL at any growth stage (Figure 2; Table 1).

Figure 2: Cambridge spray trial yield response in Einstein to 0.01ppm epi-brassinolide 2005/06
(bar labels show mean converted yield in t/ha)



Yields in Robigus were reasonable and significant differences across treatments were noted (Figure 3; Table 1). The most significant difference (at 1%) was between the control (T1) and the cumulative treatment (T7), while differences between the control (T1) and application of EBL at booting (T3), flowering (T5) and early dough stage (T6) were significant at 5%. Yellow rust was noted on Robigus at the Cambridge trial. Only the mean scores were recorded and so it was not possible to test the impact of this infection. No significant differences were noted for the green leaf area (Table 1).

Figure 3: Cambridge spray trial yield response in Robigus to 0.01ppm epi-brassinolide 2005/06
(bar labels show mean converted yield in t/ha)

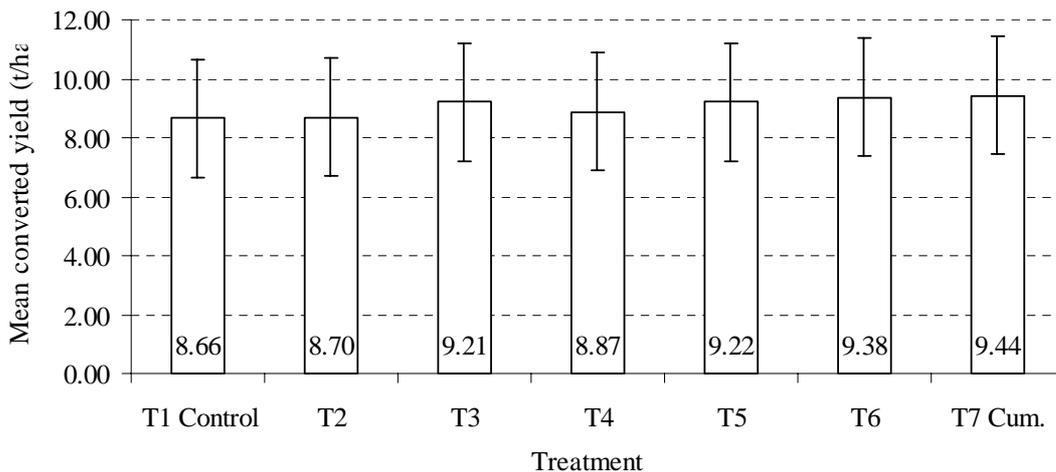
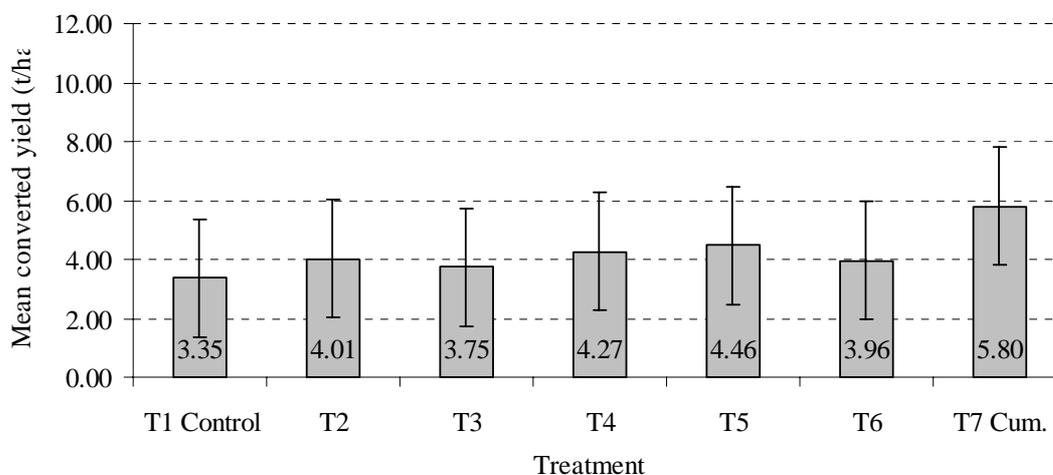


Figure 4: Cambridge spray trial yield response in Paragon to 0.01ppm epi-brassinolide 2005/06
(bar labels show mean converted yield in t/ha)



Paragon lodged significantly (Table 1) and this lowered the yield (Figure 4) relative to the other varieties in the Cambridge trial (Figure 2; 3). A significant difference (at 0.1%) was detected between the control (T1) and the cumulative EBL treatment (T7) at 0.1%.

The least significant difference of the means (LSD) for the Woolpit spray trial are reported in Table 2. No significant differences between variety, treatment or variety, treatment interaction were detected overall or between treatments within varieties. There were no lodging or disease pressures in this trial.

No visible difference in clean ripening for any of the varieties in the spray trials at Cambridge or at Woolpit was detected.

3.3.2 Seed Treatment trials

Data from the seed treatment trial at Clopton is shown in Table 3. No significant differences in the least sum differences (LSD) of the means were detected for treatment or variety or treatment, variety interaction. The replicate trial at Risby was abandoned due to poor weather so no data is available.

Table 1: Table of Least Significant Differences (LSD¹) of means across spray trial at Cambridge

Parameters	Overall trial			Einstein	Robigus	Paragon
	LSD Treatment	LSD Variety	LSD Variety x Treatment	LSD treatment	LSD treatment	LSD treatment
Yield	0.4825 **	1.4152 *	1.5442 *	0.7232 ns	0.5416 *	1.196 *
Date GS 61	-	-	-	0.989 ns	0.4549 ns	0.976 ns
Specific weight	0.770 ns	1.907 ns	2.164 ns	0.813 ns	0.5660 ns	2.177 ns
Straw length	1.917 ns	1.457 ns	3.317 ns	3.625 ns	3.807 ns	2.796 ns
Green leaf area	3.809 ns	4.142 ns	7.103 ns	3.795 ns	9.37 ns	6.151 ns
1000 grain weight	1.428 ns	2.751 ns	3.398 ns	1.859 ns	2.265 ns	3.331 ns
Lodging	6.575 ns	24.755 ns	26.010 ns	No lodging	No lodging	24.755*
Yellow rust	-	-	-	No yellow rust	Yellow rust present	No yellow rust

¹ The LSD represents the value that must be achieved between any two means being compared before the difference can be declared significant at the confidence interval indicated.

ns not significant
 * significant at 1%
 ** significant at 0.5%
 *** significant at 0.1%

Table 2: Table of Least Significant Differences (LSD¹) of means across spray trial at Woolpit

Parameters	Overall trial			Einstein	Robigus	Paragon
	LSD Treatment	LSD Variety	LSD Variety x Treatment	LSD treatment	LSD treatment	LSD treatment
Yield	0.4042 ns	0.5414 ns	0.8062 ns	0.7600 ns	0.6939 ns	0.7192 ns
Date GS 61	0.4379 ns	0.1658 ns	0.7159 ns	0.811 ns	0.7400 ns	0.803 ns
Specific weight	2.902 ns	5.994 ns	7.205 ns	5.964 ns	4.333 ns	5.187 ns
Straw length	1.869 ns	1.486 ns	3.257 ns	2.532 ns	3.936 ns	2.532 ns
Green leaf area	-	-	-	-	-	-
1000 grain weight	-	-	-	-	-	-
Lodging	No lodging	No lodging	No lodging	No lodging	No lodging	No lodging
Yellow rust	-	-	-	-	-	-

Table 3: Table of Least Significant Differences (LSD¹) of means across seed treatment trial at Clopton (ns = not significant)

Parameter	Overall trial		
	LSD Treatment	LSD Variety	LSD Variety x Treatment
Yield	0.01753 ns	0.03906 ns	0.04223 ns

3.4 Discussion

Brassinosteroids (BRs) are an important class of plant hormones, essential for plant growth and development. They are released in response stress but are also known to regulate the expression of numerous genes, including ones associated with plant height and important yield components in crops. There is little active research on the brassinosteroid pathway in the UK in the context of its potential value as an alternative source of genetic variation for yield manipulation via wheat breeding. Spraying a variety of crops with brassinosteroid preparations has been shown to increase crop establishment and yield in a number of crops. Ikekawa and Zhao (1991) in particular report that spraying wheat with an optimal dose of 0.01 ppm BR (epibrassinolide) resulted in 10-20% yield increases. The demonstration of a yield response to this hormone under UK field conditions either by direct spraying or via a seed treatment to provide preliminary evidence of the potential gains in yield that might be possible from an interaction with brassinosteroid compounds has not been conclusively provided by the pilot field trials funded during the present study.

Detection of an impact on yield following spraying with brassinosteroid in this pilot trial has proven difficult using basic and low cost field approaches. The experimental design used in these pilot field trials was such that only large varietal differences were likely to be detected and many other factors may have influenced any potential effects. In particular, many factors relating to the absorption of the chemical by the plants in the field were unknown. These would include the effectiveness of the wetting agent and climatic factors such as high temperature that may have led to evaporation of the EBL. A concentration of 0.01ppm previously reported as effective in wheat (Ikekawa and Zhao, 1991) was used, but may not have been ideal for UK conditions. Additionally, the product used in these trials was sourced as a pure chemical, rather than in a commercial formulation so may not have been ideal (stable) for field use, although it was found to be effective in lab based assays.

Although the pilot field trials produced variable results and did not conclusively highlight significant differences in varietal response, results indicated that yield loss in wheat under stress may be lower in wheat

sprayed with brassinosteroid. Trials in controlled environments using more than one concentration and using a commercial product are recommended in advance of any further field trials. Further research would also benefit from the establishment and utilisation of an absorption assay to ensure the brassinosteroid compound has been absorbed by the trial plants and that it is affecting the downstream process. After spraying, the efficacy and uptake of EBL by plants would be monitored by testing the induction of the protein *BESI* (*BR11-EMS-suppressor1*) that is a positive regulator of the BR signalling pathway. Expression of this protein is induced within an hour by spraying *Arabidopsis* with BR and continues for twenty-four hours (Yin *et al.*, 2002). The protein would be extracted from leaves harvested at appropriate intervals after spraying.

3.5 Acknowledgements

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This project was conceived by Manash Chatterjee (original project Lead Scientist at NIAB, now based at ARI in India) and Bill Angus and delivery was managed by Juno McKee (Lead Scientist from April 2006). The report was co-written by Manash Chatterjee, Bill Angus and Juno McKee.

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