RESEARCH PAPER

‘Overgrowth’ mutants in barley and wheat: new alleles and phenotypes of the ‘Green Revolution’ DELLA gene

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Abstract

A suppressor screen using dwarf mutants of barley (Hordeum vulgare L.) led to the isolation of ‘overgrowth’ derivatives, which retained the original dwarfing gene but grew at a faster rate because of a new mutation. The new mutations were in the Slender1 (Sln1) gene (11/13 cases), which encodes the DELLA protein central to gibberellin (GA) signalling, showed 100% genetic linkage to Sln1 (1/13), or were in the Spindly1 (Spy1) gene (1/13), which encodes another protein involved in GA signalling. The overgrowth mutants were characterized by increased GA signalling, although the extent still depended on the background GA biosynthesis capacity, GA receptor function, and DELLA activity. A comparison between two GA responses, α-amylase production and leaf growth rate, revealed degrees of specificity for both the overgrowth allele and the GA response under consideration. Many overgrowth mutants were also isolated in a dwarf line of bread wheat (Triticum aestivum L.) and 19 new alleles were identified in the Rht-B1 gene, one of the ‘Green Revolution’ semi-dwarfing genes and the orthologue of Sln1. The sites of amino acid substitutions in the DELLA proteins of both species provide insight into DELLA function, and included examples where identical but independent substitutions were observed. In both species, the starting lines were too dwarfed to be directly useful in breeding programmes, but new overgrowth derivatives with semidwarf heights have now been characterized. The variation they exhibit in GA-influenced traits identifies novel alleles with perfect markers that are of potential use in breeding.

Key words: barley, DELLA, gibberellins (GA), overgrowth mutant, suppressor screen, wheat.

Introduction

Many gibberellin (GA) mutants, either spontaneous in origin or identified after mutagenesis, have been characterized in a range of plant species. These include distinct dwarf and elongated (‘slender’) phenotypes that typically result from changes in either GA content or GA signalling. Identification of the genes involved and analysis of the proteins they encode have been fundamental in developing an understanding of growth regulation by GA, particularly in the model species of rice and Arabidopsis. Bioactive GAs bind to a GA receptor protein (GID1; Ueguchi-Tanaka et al., 2005; Murase et al., 2008; Shimada et al., 2008) and form a complex that is then bound by DELLA proteins, a subfamily of the GRAS transcription factor family (Griffiths et al., 2006; Willige et al., 2007; Hirano et al., 2010). The GA–GID1–DELLA complex is a target for binding of an SCF E3 ubiquitin ligase via the F-box subunit, resulting in ubiquitination of DELLA and its subsequent proteasome-mediated degradation (Fu et al., 2002; Sasaki et al., 2003; Dill et al., 2004). DELLA proteins are inhibitors of growth, and in one case the evidence suggests that DELLA binding to phytochrome-interacting factor (PIF) transcription factors effectively prevents them from promoting the expression of genes that stimulate hypocotyl growth (de Lucas et al., 2008; Feng et al., 2008).
**Materials and methods**

*Plant material*

Himalaya barley and three previously characterized dwarf mutant derivatives are described in Table 1, together with 13 new overgrowth mutants and stocks derived from backcrossing and intercrossing. Grains of tall (Rht-B1a) and dwarf (Rht-B1c) BC2 isolines of the Brazilian breadwheat variety Maringá (Hoogendoorn et al., 1988) were provided by the Australian Winter Cereals Collection, Tamworth, NSW, Australia. Plants were grown in a greenhouse in 20 cm pots containing a compost-based mix under natural light (with daylength extension to 14 h provided during winter months).

*Mutagenesis*

The method for mutagenesis of barley and wheat grains was a simplification of an earlier procedure (Zwar and Chandler, 1995). Grain (1–2 kg) of each line was imbibed in twice its mass of water at 4 °C overnight, transferred to 2 l measuring cylinders filled with water, and aerated from the bottom with pressurized air for 8 h, with one change of fresh water given after 4 h. The water was removed and grain was incubated for 2 h in freshly prepared 1 mM sodium azide dissolved in 0.1 M potassium phosphate buffer (pH 3.0), and then washed extensively in running water for 2 h, placed in a fume hood to dry overnight, and sown in the field within several days of treatment.

*Construction of derivative lines carrying overgrowth alleles*

The barley overgrowth alleles were backcrossed, intercrossed, and outcrossed to generate a set of lines suitable for detailed physiological characterization (Supplementary Fig. S1 at JXB online). Four of the new Sl1 dwarf overgrowth alleles occurred in either gsd2b or gse1n dwarfing backgrounds and were backcrossed for two generations to the WT allowing overgrowth phenotypes to be compared in tall and dwarf backgrounds. The loss of the original dwarfing allele was confirmed by PCR. The remaining seven new Sl1 overgrowth alleles occurred in the Sl1/d dwarf background, and four of these (Sl1/d.7, Sl1/d.8, Sl1/d.9 and TR103) have been through two generations of back-crossing to the WT. Two overgrowth lines (TR56 and TR216) involved identical mutations in Sl1 (G829A), but one occurred in a Sl1/d allele and the other in a Sl1 (WT) allele. These lines were intercrossed, and F2 plants were isolated that were homozygous at Sl1 (Sl1/d.7/sl1s) but lacked gsd2b (from the TR216 parent). The progeny of such heterozygotes showed a 1:2:1 ratio of genotypes, allowing the effect of sl1s on growth to be assessed in a 'tall' (sl1s/sl1s) versus 'DELLA dwarf' (Sl1/d.7/Sl1/d.7) genetic background. The spy1a allele was crossed into GA deficiency and GA receptor dwarfing backgrounds to determine whether it would enhance GA signalling.

*Leaf elongation rates and GA dose–response curves*

The methods used have been described previously (Chandler and Robertson, 1999). Curves were fitted to data points using a four-parameter Hill equation.

*α-Amylase production by endosperm half-grains*

Endosperm half-grains were prepared and incubated with or without gibberellic acid (GA3; 1 μM) at 22 °C for 0, 42, or 72 h. To each sample, 1.5 ml of a solution of 10 mM CaCl₂ was added, the half-grains were homogenized, and an aliquot of 1 ml was clarified by centrifugation (20 000g for 5 min). The supernatant was analysed for α-amylase activity using the Megazyme α-amylase (Ceralpha) procedure.

*Coleoptile lengths of barley overgrowth lines*

Coleoptile lengths were determined on seedlings after 21 d of growth in the dark with a daily temperature programme of 12 h at 12 °C and 12 h at 8 °C.

*PCR amplification of DNA and sequencing*

DNA was prepared from leaf material of barley and wheat lines by the method of Ellis et al. (2005). PCR amplification of barley sequences used primers specific for the Sl1, Spy1, and Gse1 genes.
maturity ranged from intermediate between the dwarf parent and the presence of a dwarfing allele. The degree of growth enhancement was confirmed by sequencing the appropriate PCR-amplified wheat sequences were amplified using primer pairs in which one primer was specific for the Rht-B1 gene (Supplementary Table S1 at JXB online). The 3' half of the gene in an initial selection of 60 overgrowth mutants was amplified using conserved forward primers but reverse primers that were specific to B gene sequences in the 3' untranslated region.

Results
Isolation of overgrowth mutants in barley

The starting material comprised three dwarf derivatives of Himalaya barley, each representing a defined single-nucleotide substitution affecting GA biosynthesis (GA 3-oxidase, Grd2), the GID1 GA receptor (Gse1), or DELLA (Sln1). Grains of each dwarf line were treated with sodium azide, sown in the field for harvest of M2 grains, and M2 seedlings growing in soil were screened at the second-leaf stage for those that showed more rapid growth than their dwarf siblings (1 x 10^6 grains sown, representing about 50 000 M1 spikes).

Of most interest were 22 plants (Fig. 1), recovered across all three dwarf backgrounds, that were fully fertile and had progeny that were uniform and showed enhanced growth. In each case, the presence of the original dwarfing mutation was confirmed by sequencing the appropriate PCR-amplified fragment. We refer to these as ‘overgrowth’ mutants, as their leaf elongation rate was higher than expected based on the presence of a dwarfing allele. The degree of growth enhancement differed among lines (see below), and their height at maturity ranged from intermediate between the dwarf parent and WT to as tall as the WT.

Identification of mutations and genetic linkage studies

The Sln1 gene was sequenced in DNA from the overgrowth mutants, as this is one of several candidate genes where mutation might lead to an overgrowth phenotype. New mutations in the Sln1 ORF were found in 20 of the 22 lines, and they defined 11 new Sln1 alleles (some lines carried identical mutations and may have been sibs): seven occurred in the Sln1d dwarf background (and were hence intra-allelic mutations and may have been sibs); seven occurred in the Sln1d dwarf background.

Fig. 1. Screening for overgrowth mutants in barley. One tall seedling among dwarf siblings. The pale yellow seedling (front left) is an example of the 2–3% of non-green seedlings that are seen in barley M2 populations.

The LERmax value achieved by the first leaf under standard conditions is a robust measure of GA response (Chandler and Robertson, 1999) and was determined for all of the overgrowth lines and their parents (Table 2). The 13 independent
overgrowth lines all had significantly higher LER\textsubscript{max} values than their respective dwarf parent, and the extent of growth enhancement varied in an allele-dependent manner. Three overgrowth alleles (\textit{sln1m}, \textit{sln1n}, and \textit{sln1s}) were compared in both their original dwarfing background and after backcrossing to the tall WT background; growth rates were consistently lower in dwarf backgrounds, indicating that overgrowth alleles are still subject to decreased GA signalling resulting from either impaired GA biosynthesis or GA receptor function. In a WT background, the overgrowth alleles generally enhanced growth rates (additional data presented below). Identical nucleotide substitutions in \textit{sln1s} and \textit{Sln1d.7} allowed us to assess whether impaired DELLA function affected growth promotion by the overgrowth allele \textit{sln1s}. These two overgrowth alleles are of independent origin, and differ in whether the \textit{Sln1d} mutation is also present. The M244seg stock segregates tall (\textit{sln1s/sln1s}) and dwarfed (\textit{Sln1d.7/Sln1d.7}) lines (see Materials and methods) whose LER\textsubscript{max} values were determined as 35.1±0.8 and 12.9±0.4 mm d\textsuperscript{-1}, respectively, compared with dwarf \textit{Sln1d} at 8.3±0.3 mm d\textsuperscript{-1}. We concluded that \textit{sln1s} is subject to impaired DELLA activity. Taken together, these results highlighted a distinctive difference between the new \textit{Sln1} overgrowth alleles and typical loss-of-function elongated \textit{sln1} alleles such as \textit{sln1c}; the former showed lower growth rates when GA signalling was impaired by upstream mutations affecting GA biosynthesis or signalling (Chandler and Robertson, 1999), whereas the latter showed constitutive growth rates.

**Fig. 2.** Distribution of LER\textsubscript{max} values of individual F\textsubscript{2} seedlings from different crosses. Values for parents (mean ± standard error) were WT, 35.2±1; \textit{Sln1d}, 7.6±0.4; \textit{Sln1d.5}, 13.7±0.4; \textit{Sln1d, spy1a}, 17.5±0.4; TR103, 16.6±0.6.
Table 2. Leaf elongation rates and α-amylase production of overgrowth mutants. For results for TR26, see Table 3. ND, not determined.

| Line   | Genotype | LER\(_{\text{max}}\) (mm d\(^{-1}\)) | α-Amylase activity (Ceralpha units/grain) |
|--------|----------|---------------------------------------|------------------------------------------|
|        |          |                                       | 0 h (×10\(^3\)) | 42 h | 72 h |
| Him    | WT       | 34.8±0.9                              | 1.6 | 0.36±0.08 | 1.7±0.09 |
| Him (GA\(_3\)) | WT     | 52.3±0.1                              | ND | 9.81±0.86 | 22.1±2.8  |
| M640   | Sln1d   | 9.2±0.2                               | 1.6 | 0.21±0.01 | 0.19±0.05 |
| M640 (GA\(_3\)) | Sln1d | 12.1±0.3                              | ND | ND      | ND      |
| TR1    | Sln1d.4 | 23.8±0.5                              | 2.5 | 0.26±0.01 | 2.88±0.27 |
| TR9    | Sln1d.5 | 15.5±0.7                              | 1.6 | 0.21±0.01 | 0.16±0.04 |
| TR13   | Sln1d.6 | 15.8±0.6                              | 1.8 | 0.20±0.02 | 0.19±0.04 |
| TR56   | Sln1d.7 | 16.7±0.6                              | 2.7 | 0.93±0.22 | 3.24±0.47 |
| TR60   | Sln1d.8 | 28.6±0.9                              | 3.6 | 0.82±0.13 | 3.76±0.23 |
| TR100  | Sln1d.9 | 20.2±0.6                              | 3.2 | 4.34±0.13 | 14.6±0.39 |
| TR103  | ND      | 16.1±0.4                              | 3.4 | 0.74±0.10 | 0.39±0.1  |
| TR107  | Sln1d.10| 13.2±0.3                              | 2.4 | 0.39±0.07 | 0.17±0.03 |
| M463   | grd2b   | 16.9±0.8                              | 2.5 | 0.67±0.04 | 1.3±0.2   |
| M463 (GA\(_3\)) | grd2b | 49.8±0.8                              | ND | 7.20±0.73 | 18.9±1.4  |
| TR216  | grd2b, sln1s | 23.2±0.6          | 4.1 | 1.01±0.12 | 4.16±0.55 |
| TR261  | grd2b, sln1m | 38.4±1.5          | 3.8 | 7.50±0.24 | 18.1±0.8  |
| TR305  | grd2b, sln1o | 32.9±0.9          | 3.8 | 9.85±0.43 | 16.9±1.2  |
| M693   | gse1n   | 19.7±0.4                              | 2.5 | 0.29±0.05 | 0.11±0.01 |
| M693 (GA\(_3\)) | gse1n | 35.3±1.2                              | ND | ND      | ND      |
| TR407  | gse1n, sln1n | 29.3±0.5          | 9.9 | 0.27±0.03 | 0.26±0.13 |
| M240   | sln1m   | 42.2±1.6                              | 6.5 | 11.6±1.4 | 22.6±2.3  |
| M242   | sln1n   | 44.3±3.1                              | 3.0 | 1.07±0.08 | 6.39±1.52 |
| M243   | sln1s   | 35.4±0.9                              | 3.8 | 3.66±0.53 | 15.4±0.9  |

The spy1a overgrowth allele enhances growth of GA biosynthesis and GA receptor dwarfs

The mutant spy1a overgrowth allele was backcrossed into GA biosynthesis and GA receptor dwarf backgrounds, as well as to the WT (Table 3). The presence of spy1a significantly increased growth rates of the dwarf lines, although not to WT values (Table 3). In a WT background, spy1a caused only marginal increases in growth rate, at the borderline of statistical significance when different experiments and different grain harvests were assessed (Table 3). We concluded that spy1a enhanced growth in all three dwarfing backgrounds but only to a limited extent, indicating that growth rates were still restricted by impaired GA signalling, as found for the Sln1 overgrowth alleles.

α-Amylase production by endosperm half-grains of Sln1 and Spy1 overgrowth mutants

The production of α-amylase by endosperm half-grains of barley is typically dependent on the presence of an active GA. Therefore, monitoring α-amylase activity in the absence of an active GA provides a convenient measure of the extent of basal GA signalling. Two control lines with normal GA sensitivity (WT and grd2b) showed a near 15-fold increase in α-amylase activity over basal levels after 72 h incubation with GA\(_3\) (Table 2). When overgrowth mutants and their dwarf parent were examined, the initial amount of α-amylase activity in mature endosperm half-grains was very low (Table 2, 0 h, note values are ×10\(^3\)), but, with incubation some overgrowth lines (Sln1d.4; Sln1d.7; Sln1d.8; Sln1d.9; grd2b, sln1m; grd2b, sln1o; and grd2b, sln1s) showed enhanced production of α-amylase relative to their dwarf parent, whereas others (Sln1d.5; Sln1d.6; Sln1d.10; and gse1n, sln1n) did not. Among the overgrowth derivatives of Sln1d, the Sln1d.9 derivative was exceptional, accumulating very high levels of α-amylase at both 42 and 72h incubation, despite this line showing only a modest restoration of growth rate (Table 2).

Overgrowth alleles backcrossed into a tall background had higher rates of α-amylase accumulation than the original dwarf lines; for instance, the increase in α-amylase production by sln1s was higher in the WT background (M243) than in either Sln1d (TR56) or grd2b (TR216) dwarf backgrounds. Similar results were noted for sln1n (M242 vs TR407) and possibly sln1m (M240 vs TR261). This observation was comparable to those above for growth rates.

The extent of two different GA responses (growth rate and α-amylase production, both in the absence of GA) was plotted for each overgrowth line as a percentage of the WT+GA value (Fig. 3). Selected examples that illustrate marked differences in the extents of the two responses compared with the parent line are indicated. Sln1d.9 was clearly different from Sln1d and the other Sln1d-derived overgrowth lines (Fig. 3, circles) in its high levels of α-amylase and relatively slow growth rate. Other comparisons revealed similar differences; the sln1o allele in a grd2b background had greatly increased α-amylase accumulation relative to its parent (Fig. 3, squares); the sln1n allele in a gse1n background grew more rapidly than its parent (Fig. 3, diamonds), although α-amylase accumulation was similar; and the backcrossed lines sln1m and sln1n...
Chandler and Harding had similar growth rates to WT (triangles) but differed greatly in their extent of α-amylase accumulation. Overall, these results illustrated examples of large differences between Sln1 overgrowth alleles in two defined GA responses, indicating that the effect of an overgrowth allele in enhancing GA signalling depends on both the allele and the GA response being investigated.

The spy1a allele showed much higher levels of α-amylase production in both WT and dwarf backgrounds than in the original Sln1d dwarfing background (Table 3). This behaviour contrasts with its much smaller effects on growth rate (Table 3), and the conclusions reached above on the GA response specificity of Sln1 overgrowth alleles also extend to spy1a.

**GA responsiveness of overgrowth lines**

The LER max values (Tables 2 and 3) indicated that overgrowth alleles enhance leaf elongation rates of both dwarf and tall genotypes but are still influenced by the extent of GA signalling. To investigate this in more detail, we determined GA dose–response growth curves for each overgrowth allele. The curves for four selected alleles are shown in Fig. 4, and three general observations could be made. First, the overgrowth alleles conferred enhanced growth rates across all concentrations of applied GA in dwarf backgrounds, and generally did so in a WT background as well, although at high GA concentrations the effect was small or even non-existent. Secondly, overgrowth derivatives of GA signalling dwarf lines (Sln1d, gse1n) never fully restored their growth rate to that of the WT, even at high GA concentrations (Fig. 4A–C), in contrast to the GA biosynthetic dwarf background (Fig. 4D). Thirdly, most of the transition in growth rates in overgrowth derivatives of lines with normal GA signalling occurred over the GA concentration range of 0.01–1 μM, the same as that identified previously for GA-deficient mutants (Chandler and Robertson, 1999). This indicated that overgrowth alleles do not markedly change their ‘sensitivity’ (H50) for growth responses to GA. These results confirmed that overgrowth alleles enhanced the degree of GA signalling in a manner that was influenced by different genetic backgrounds and by the concentration of exogenous GA.

**Other traits associated with overgrowth alleles**

The overgrowth lines were close to normal in appearance during growth and at maturity, apart from differences in overall height. None of the nine overgrowth derivatives of Sln1d was as tall as the WT at maturity, in contrast to the remaining four overgrowth lines, which were as tall as the WT. Coleoptile lengths of overgrowth lines (Supplementary Table S2 at JXB online) varied in general accordance with LER max values and with final plant height.

A general feature of overgrowth lines is that they produce larger grains than their dwarf parent. In different harvests from different growing seasons, grain sizes were generally intermediate between the parental dwarfs (grain mass about 40 mg) and the tall WT (grain mass about 55 mg). In different greenhouse generations, the grains of grd2b,shl1m were on average 40% larger than those of the grd2b dwarf parent. When backcrossed to Himalaya, the shl1m allele resulted in an average 20% increase in grain size. Selected overgrowth

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**Table 3. Effect of spy1a on growth rates and α-amylase production.**

| Line    | Genotype       | LER max (mm d⁻¹) | α-Amylase activity (Ceralpha units/grain) |
|---------|----------------|-----------------|------------------------------------------|
|         |                |                 | 0h ×10⁻³ | 42 h | 72 h       |
| Himalaya WT | 34.6±0.9    | 1.6             | 0.36±0.08 | 1.7±0.09 |
| M247 spy1a   | 37.6±0.7    | 4.6             | 3.33±0.22 | 12.5±1.2 |
| M640 Sln1d   | 9.2±0.2     | 1.6             | 0.21±0.01 | 0.19±0.05 |
| TR26 Sln1d, spy1a | 17.1±0.4 | 2.3             | 0.66±0.10 | 2.84±0.46 |
| M463 grd2b   | 17.7±0.6    | 2.1             | 0.53±0.1  | 3.7±0.3  |
| M248 grd2b, spy1a | 23.4±0.5 | 3.6             | 10.2±0.9  | 15.0±1.8 |
| M691 gse1n   | 20.6±0.4    | 1.6             | 0.07±0.01 | 0.13±0.03 |
| M249 gse1n, spy1a | 25.8±0.5 | 2.1             | 1.2±0.2   | 7.2±1.0   |
alleles have been backcrossed into the commercial barley variety ‘Sloop’, and a full analysis on grain size and other traits will be made on BC3 sister lines.

Overgrowth mutants in wheat

Grains of an Rht-B1c dwarf in a var. ‘Maringá’ background were treated with sodium azide, sown in the field, and M2 plants were screened for overgrowth mutants either during early growth or at maturity in the field. From these two screens (1.6 million M2 plants), approximately 400 plants were selected and characterized either by rapid early leaf elongation or by increased height at maturity. As seen for barley, there was a wide range among different plants in their degree of growth restoration (Fig. 5).

We sequenced the portion of the wheat Rht-B1 gene that corresponds to the region of Sln1 in barley where overgrowth mutations were identified. From an initial selection of 60 wheat overgrowth lines, we identified 19 new derivative alleles of Rht-B1c, which were named using the same convention as for overgrowth derivatives of Sln1d barley, i.e. Rht-B1c.1, Rht-B1c.2, Rht-B1c.3, and so on (Table 4). These included two different classes of mutation responsible for the overgrowth phenotype. Four alleles (Rht-B1c.11, Rht-B1c.13, Rht-B1c.19, and Rht-B1c.20) were premature termination codons, which in barley result in an elongated slender phenotype and male sterility. These four wheat alleles had growth and fertility similar to that of the tall isolate; presumably the expression of the A- and/or D-genome DELLA proteins provides genetic compensation for the B-genome null mutant.

The remaining alleles identified 15 new amino acid substitutions in the wheat DELLA protein encoded by the B-genome, which were associated with varying degrees of growth recovery (Table 4). It is of interest to compare these substitutions with those identified in barley.
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with those found in barley (Fig. 6). Across the two species, there were 26 single amino acid substitutions, including four sites where identical amino acid changes occurred. We interpreted this to mean that there is a moderately limited set of amino acid substitutions that will lead to an overgrowth phenotype. This conclusion was supported by the observation that within both wheat and barley there were identical but independent mutations observed.

The sites of amino acid substitutions in the C-terminal half of barley and wheat DELLA proteins are shown in relation to conserved motifs that have been previously identified (Fig. 6). The fact that overgrowth mutations were distributed throughout much of the C-terminal half provides considerable potential for alterations in the binding of DELLA to interacting protein partners.

Discussion

Overgrowth mutants of barley and wheat were isolated following mutagenesis of dwarf lines; they retained the original dwarfing alleles but grew faster than expected because of newly induced mutations. They were characterized by enhanced GA signalling, although the extent was specific for both the allele and the GA response being considered. Previous studies in Arabidopsis employed suppressor screens to identify or confirm four genes with important roles in GA signalling – SPY, SLY, and the two Della genes GAI and RGA. Our results indicated that the Della genes (Sln1 in barley, Rht-1 in wheat) are the most frequent sites of overgrowth mutations. In only a single case was a different gene implicated – one of the barley overgrowth mutants was due to a new mutation in Spy1. Earlier studies on this gene indicated that it acted as a negative regulator of GA signalling in barley (Robertson et al., 1998), although its importance in GA regulation in vivo could not be assessed because no mutants were available. Now, the isolation of the spy1a mutant and the demonstration that it enhances GA signalling in a range of dwarf backgrounds as well as in the WT confirms the earlier findings, and extends the functional importance of SPY1 from Arabidopsis to cereals. The fact that suppressor screens in diverse species have yielded mutations in very similar genes indicates functional conservation of GA responses across a wide taxonomic distance. A similar screen in rice has now extended the list of genes to GID1, the GA receptor (Yamamoto et al., 2010).

Five independent lines of evidence supported the conclusion that the new mutations identified here were responsible for overgrowth phenotypes, rather than being coincidental effects of prior treatment with mutagen. First, for each of the 13 barley mutants, a ‘control’ gene (Gse1) of about the same length as Sln1 was sequenced, and in no case was a nucleotide substitution detected (data not shown). Secondly, the mutations identified were almost (30/31) exclusively G → A (assuming C → T represents G → A in the opposite strand). The redundancy in the genetic code allows 33% of random G → A
changes to have no corresponding amino acid substitution; however we did not observe a single case of silent substitution. Thirdly, the mutations we identified involved amino acid residues that are very highly conserved; in all but two cases (both involving very conservative amino acid substitutions), the DELLA mutants involved amino acid residues that are identical between cereal species and the taxonomically distant Arabidopsis. Substitution of highly conserved amino acid residues is much more likely to result in disruption of protein function than changes in poorly conserved residues. Fourthly, independent mutagenesis treatments gave examples of identical mutations induced within barley, between barley and wheat, and within wheat (see Results). This is highly unlikely to occur by chance, and instead suggests that there is a moderately limited spectrum of mutations that will generate an overgrowth phenotype. Fifthly, we always observed 100% linkage between mutant phenotypes and amino acid sequences following crossing and subsequent segregation in both barley and wheat.

Overgrowth alleles enhance GA signalling and so are most likely to cause reductions in either the amount of DELLA protein or its ‘activity’, the latter probably involving interactions with other proteins. We think it most likely that mutant DELLA proteins have reduced affinity for interacting protein partners, resulting in a greater promotion of ‘GA’ responses. The barley and wheat DELLA proteins share 95% amino acid sequence identity, and in both species a considerable number of amino acid substitutions were in the LHR1 motif, a region that in Arabidopsis is involved in interactions with PIF4 and PIL5 (de Lucas et al., 2008; Feng et al., 2008). Mutations in this region will presumably show reduced affinity for PIF orthologues in cereals. There are also mutations elsewhere in the DELLA protein, including the VHIID and PFYRE motifs, which have substantial effects on growth but which are far removed from the currently identified LHR1 motifs important for PIF interaction. Very little is known about protein partners that interact with the PFYRE motif, or with DELLA proteins in general for other GA responses such as α-amylase production and grain dormancy. The differential effects of particular overgrowth alleles on growth versus α-amylase production in barley suggest that different regions of the DELLA protein interact with different protein partners to regulate these two responses.

The starting lines used in this study are too dwarfed for breeding purposes and were selected for basic/strategic studies on the role of GA in growth regulation. The overgrowth lines generated from these mutants are much closer to ideotypes (e.g. semi-dwarfs) that might be useful in breeding. These are more difficult to isolate in a direct forward screen because typically they fall within the range of variation (largely non-genetic) present in the original tall parent. The identification of new Shn1 overgrowth alleles that are fully fertile and with a range of growth rates both faster and slower than the WT indicates that the previous designation of Shn1 mutants into slender DELLA and dwarf DELLA was an oversimplification, reflecting extremes at the ends of a continuum.

Barley has advantages over wheat for mutational studies, being diploid instead of hexaploid. Typical loss-of-function phenotypes are expected in barley, but not in wheat if homoeologous genes are equally expressed. Our success in isolating many new mutants in wheat stems from the fact that the dwarfism due to semi-dominant alleles such as Rht-B1c is effectively a diploid trait, involving only one of the three genomes, which allows selection of a range of loss-of-function intra-allelic mutations.

In both species new alleles were recovered for an agronomically important trait, plant height. The large collection of wheat mutants is expected to reveal many new semi-dwarfing alleles in the Rht-B1 gene, with the potential to extend the range of dwarfism (more or less dwarfed) beyond that of the existing Rht-1 semi-dwarfing genes. These might be useful in targeting specific alleles to specific environments (Flintham et al., 1997); for instance, stronger (more dwarfing) alleles in cool, moist, high-input environments and weaker alleles in warm, dry, low-input environments. But in addition, variation was also observed in other GA-influenced traits, such as grain size and grain dormancy, which are of practical interest. The dwarf Rht-B1c line has considerably higher grain dormancy than either the tall or semi-dwarf (Rht-B1b) isolines. Initial experiments on the new semi-dwarf overgrowth derivatives indicate that some have higher grain dormancy than Rht-B1b and may therefore be of value in contributing to sprouting tolerance. Furthermore, it has recently been shown that DELLA proteins influence the resistance of both wheat and barley to fungal pathogens (Saville et al., 2012). Among the many new Della alleles, we hope to find examples where suitable height is associated with other useful traits. As a single gene character, and with a perfect molecular marker, such alleles can readily be introduced into breeding programmes.

Supplementary data

Supplementary data are available at JXB online.

Supplementary Table S1. Primers for sequencing the 3′ half of wheat Rht-B1c.
Supplementary Table S2. Coleoptile lengths of barley overgrowth lines.
Supplementary Fig. S1. Barley seedlings of different genotypes at the same age.
References

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