Agronomic traits affected by dwarfing gene Rht-5 in common wheat (Triticum aestivum L.)

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**Abstract**

Investigating the genetic effects of dwarfing gene *Rht*-5 on plant height and other agronomic traits in common wheat is important for its proper use in wheat breeding program. In this study, the effects of dwarfing *Rht*5 on agronomic traits in wheat were evaluated in the F\(_2\) population and forty F\(_2\);F\(_3\) lines derived from a cross between Ningchun10 (*rh5*) and Marfed M (*Rht5*) along with the two parents (Ningchun10 and Marfed M). The genotypes of F\(_2\) individuals were identified using SSR marker BARC 102 linked with *Rht5*, and the dwarf and tall individuals were selected to compose the F\(_2\);F\(_3\) lines. Analysis of variance in the F\(_2\) population and F\(_2\);F\(_3\) lines indicated highly significant differences among individuals or lines for all of traits studied, except for biomass, grain yield and harvest index. In general, dwarfing gene *Rht*-5 was associated with a plant height reduction of 23.16\%, delaying heading date and maturity, increasing the number of fertile tillers plant\(^{-1}\), while reducing the number of spikelets spike\(^{-1}\) and number of grains spike\(^{-1}\). Earlier heading date, longer spike, more spikelets spike\(^{-1}\), and more grains spike\(^{-1}\) could be observed in the dwarf lines when compared to the dwarf parent. Plant height showed positive and highly significant correlation with peduncle length (0.86), spike length (0.76) and the dwarf and tall individuals were selected to compose the F\(_2\);F\(_3\) lines for all of traits studied except for biomass, grain yield and harvest index. The results obtained could be helpful for the proper use of dwarfing gene *Rht*-5 in breeding programs to improve lodging tolerance and yield potential in wheat.

**Keywords:** Common wheat (*Triticum aestivum* L.), *Rht*-5 dwarfing gene, Plant height.

**Abbreviations:** GAR_Gibberellic acid-responsive; GAI_Gibberellic acid insensitive; SD_Standard deviation.

**Introduction**

Wheat (*Triticum aestivum* L.) is the primary source of staple diet for poor and rich alike and it is the leading food in many areas of the world. It provides 20% food calories to the world. With the global population growing and arable land limited, wheat production and yield improvement become even more important (Rajaram, 2002). Since the Green revolution, semi-dwarf wheat varieties have been emphasized in most wheat improvement programs (Singh et al., 2001), and they have replaced the old tall wheat mostly in irrigated and high yielding regions of the world (Byerlee and Moya, 1993).

Those dwarfing and semi dwarfing genes have played important roles in reducing plant height, increasing harvest index, improving lodging resistance, and increasing grain yield. Plant height is an important consideration for many wheat growers and plant breeders developing cultivars to meet grower needs (Budak et al., 1995; Baenziger et al., 2004a). Reduced plant height is the key objective of wheat breeding programs worldwide (Mathews et al., 2006). Most current wheat varieties contain *Rht*-B1b (formerly *Rht1*) or *Rht*-D1b (formerly *Rht2*), which were transferred from the Japanese variety ‘Norin10’ into a wide range of CIMMYT germplasm before being taken up by other wheat breeding programs worldwide (Gale et al., 1985). By conferring insensitivity to gibberellic acid (GA), these genes have pleiotropic effects on plant growth, causing reductions in coleoptile length and seedling leaf area (Allan et al., 1962; Whan, 1976; Rebetzke et al., 2001). There is an increasing interest in the development of wheat cultivars with greater seedling vigor and the capacity to emerge from deep sowing (Rebetzke and Richards, 2000; Schillinger et al., 1998).

Replacement of the *Rht*-B1b and *Rht*-D1b GAR-dwarfing alleles with alternate GA-responsive (GAR) dwarfing genes shows great potential for reducing plant height without compromising seedling vigor (Rebetzke and Richards, 2000; Rebetzke et al., 1999, 2004a; Ellis et al., 2004). Indeed, studies have already demonstrated the potential of GAR dwarfing gene *Rht8* in the development of semi-dwarf, long-coleoptile wheat targeted at sowing depths exceeding 100 mm (Schillinger et al., 1998; Rebetzke et al., 2007b). *Rht*8 has a smaller effect on height reduction (ca. 8–12\%) than the GAR-dwarfing genes *Rht*-B1b, *Rht*-B1c and *Rht*-D1b (Rebetzke and Richards, 2000; Ellis et al., 2004, 2005). Despite this, *Rht*8 has been identified in commercial wheat varieties (Zhang et al., 2006), highlighting the utilization of the GAR-dwarfing genes in breeding of commercial varieties. The *Rht*8 allele has been shown to reduce plant height and increase carbon-partitioning to grain to increase grain number and yield (Rebetzke and Richards, 2000). In addition to *Rht*8, there is a suite of major GAR dwarfing genes (e.g. *Rht*4, *Rht*5, *Rht*12, *Rht*13, *Rht*14, and *Rht*18) that reduce plant height by as much as 50\% when compared with tall-parental or near-isogenic controls (Loskutova, 1998; Ellis et al., 2004, 2005).
but are seemingly neutral in their effects on coleoptile length and seedling leaf size (Ellis et al., 2004). As yet, there has been only a small amount of work (Loskutova, 1998, Rebetzke et al., 2000; Ellis et al., 2004) investigating the potential yield improvement offered by these loci, or their effects on other important traits. The Rht-5 dwarfing gene was dominant, and associated with molecular marker Xbarc102 on chromosome 3BS with estimated genetic distance of approximately 10 cM (Ellis et al., 2005). Rht5 has a greater effect on height reduction (-55%) than Rht12 (-45%), Rht13 (-34), Rht4 (-17), and Rht8 (-7%) (Rebetzke et al., 2012). This study is aimed to better understand the genetic effects of dwarfing Rht-5 on plant height and other agronomic traits in common wheat.

Results

The effects of Rht5 on agronomic traits in F2 population

The mean values along with standard deviation (SD) for the tall and dwarf group, the mean differences between the tall and dwarf group, and the effects of dwarfing gene Rht5 on the agronomic traits in the F2 population were estimated and showed in Table 1. The highest values for standard deviation in the dwarf group were observed for plant height (9.36), number of grains spike^{-1} (9.10), and number of fertile tillers plant^{-1} (9.07), which suggested, greater magnitude of variability among the F2 population for these traits and the potential for selection of good lines with better agronomic traits and semi-dwarf architecture. The plant height of the tall group was reduced by 23.91% in the dwarf group, which indicated that dwarfing gene Rht-5 could greatly reduce the plant height. The tall group headed about 10 to 24 days earlier than that of the dwarf group, while there was no significant difference between tall and dwarf group in the number of fertile tillers. These findings suggested that delaying heading date was one of the drawbacks of dwarfing gene Rht5, but the effects could be reduced during the selection. The spike length of the tall group was significantly longer than that of the dwarf group by 21.16%. The number of spikelets spike^{-1} and number of grains spike^{-1} of the tall group were more than that of the dwarf group by 11.51% and 38.1%, respectively.

Detection of genotypes by SSR marker

Amplification with primer BARC102 showed polymorphism both in parents and in the F2,3 progeny. Two clear polymorphic bands were amplified by primer BARC102 with each one differentiating between the two parents. As can been seen from figure 1, differences in the BARC102 genotype showed a 175-bp product in Ningchun10 (rht5, signed as P1 in Fig1) and a 200-bp in Marfed M (Rht5, marked as P2 in Fig1), indicating polymorphism both in the parents and in the F2,3 lines. Thus, the F2,3 lines of Ningchun10 x Marfed M could be classified as tall (rht5) or dwarf (Rht5) as Rht5 was dominant and the heterozygous genotype performed as the dwarf phenotype. Classifying the individuals into two groups based on the presence or absence of the polymorphic band lead to 16 individuals as dwarf

Table 1. Means, ranges, standard deviations and mean differences for measured traits between Tall (43) and Dwarf (91) individuals among the F2 population of Ningchun10/Marfed M and the estimated effects of Rht5.

<table>
<thead>
<tr>
<th>Genotype class</th>
<th>Tall (rht5)</th>
<th>Dwarf (Rht5)</th>
<th>Difference</th>
<th>Effect (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean±SD</td>
<td>Range</td>
<td>Mean±SD</td>
<td>Range</td>
</tr>
<tr>
<td>Plant height(cm)</td>
<td>98.77±7.52</td>
<td>90-124</td>
<td>75.15±9.36</td>
<td>53-89.8</td>
</tr>
<tr>
<td>Heading date (d)</td>
<td>212.67±5.49</td>
<td>185-220</td>
<td>218.23±5.96</td>
<td>209-230</td>
</tr>
<tr>
<td>No. fertile tillers plant^{-1}</td>
<td>20.60±9.77</td>
<td>6-39</td>
<td>16.47±9.07</td>
<td>2-44</td>
</tr>
<tr>
<td>Spike length(cm)</td>
<td>11.25±2.09</td>
<td>9.3-23</td>
<td>8.87±1.21</td>
<td>5.6-12</td>
</tr>
<tr>
<td>Spikelets No. spike^{-1}</td>
<td>21.28±1.40</td>
<td>18-25</td>
<td>18.3±2.15</td>
<td>14-27</td>
</tr>
<tr>
<td>Grains No. spike^{-1}</td>
<td>40.58±11.10</td>
<td>11-64</td>
<td>25.12±9.10</td>
<td>6-54</td>
</tr>
</tbody>
</table>

Fig 1: Bands showing polymorphism between parents and 14 F2,F3 lines

The arrows indicate polymorphic bands (tall and dwarf lines); P1: Ningchun10 (tall parent), P2: Marfed M (dwarf parent).
Table 2. Analysis of variance of agronomic traits evaluated among the 40 F_{2:3} lines of common wheat.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Genotypes</th>
<th>Replications</th>
<th>Error</th>
<th>F value</th>
<th>C.V (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plant height</td>
<td>580.58**</td>
<td>98.58</td>
<td>68.32</td>
<td>8.50</td>
<td>7.42</td>
</tr>
<tr>
<td>Peduncle length</td>
<td>69.78**</td>
<td>31.11</td>
<td>16.63</td>
<td>4.20</td>
<td>10.81</td>
</tr>
<tr>
<td>Heading date</td>
<td>14.86**</td>
<td>8.68*</td>
<td>1.29</td>
<td>11.53</td>
<td>0.55</td>
</tr>
<tr>
<td>No. fertile tillers plant</td>
<td>48.72*</td>
<td>42.06</td>
<td>27.52</td>
<td>1.77</td>
<td>27.19</td>
</tr>
<tr>
<td>Spike length</td>
<td>2.63**</td>
<td>2.093</td>
<td>0.695</td>
<td>3.77</td>
<td>8.29</td>
</tr>
<tr>
<td>Spikelets No. spike¹</td>
<td>3.87**</td>
<td>2.73</td>
<td>1.28</td>
<td>3.01</td>
<td>5.46</td>
</tr>
<tr>
<td>Grains No. spike¹</td>
<td>116.49**</td>
<td>143.18</td>
<td>54.82</td>
<td>2.13</td>
<td>19.64</td>
</tr>
<tr>
<td>Biomass</td>
<td>89.34</td>
<td>10.74</td>
<td>67.696</td>
<td>1.32</td>
<td>34.15</td>
</tr>
<tr>
<td>Grain yield</td>
<td>12.21</td>
<td>0.46</td>
<td>9.26</td>
<td>1.32</td>
<td>42.82</td>
</tr>
<tr>
<td>harvest index</td>
<td>0.0047</td>
<td>0.0096</td>
<td>0.0042</td>
<td>1.13</td>
<td>22.19</td>
</tr>
</tbody>
</table>

** Significant at p<0.01, *Significant at p<0.05.

The analysis of variance showed highly significant difference among all the F_{2:3} lines for all the traits investigated, suggesting genetic differences existed among those F_{2:3} lines except for biomass, grain yield and harvest index (Table 2). The progenies exhibiting superior values in most of the desired yield components suggested that selection could be conducted for further evaluation in advanced segregation generations, even in advanced yield trial and adaptability studies (Camargo et al., 2000; Ansari et al., 2005). The genetic variability of number of grains spike¹ (19.64%) and number of fertile tillers plant¹ (27.19%) were the highest compared to the other traits (Table 1). The coefficients of variation of plant height, spike length, peduncle length and number of spikelets spike¹ were 7.42%, 8.29%, 10.81 and 5.46%, respectively, indicating moderate genetic variability of these traits.

Agronomic comparison of tall and dwarf parent

The investigation on the two parents indicated that the plant height and peduncle length of tall parent Ningchun10 were 140.75 cm and 48.28 cm, respectively, which were taller and longer than that of the Rht5 donor Marfed M by 45.1% and 48.59% (Table 3, Fig 2), respectively. The tall architecture made almost all of tall parent lodged at the grain-filling stage, but there was no lodging in dwarf parent. The heading date of the tall parent was about 8 days earlier than that of the dwarf parent. The number of fertile tillers plant¹ of the dwarf parent was more than that of tall parent by 107.71%. The spike length of the tall parent was longer than that of the dwarf parent by 46.28%. The number of spikelets spike¹ and number of grains spike¹ of the tall parent were more than that of the dwarf parent by 46.28% and 46%, respectively, which indicated that the tall parent had greater yield potential than the dwarf parent.

Fig 2: Part of the field experiment showing parental genotypes with different plant height.
P1: Ningchun10 (tall parent), P2: Marfed M (dwarf parent)
The effects of Rht5 on agronomic traits in F<sub>2,3</sub> lines

The mean values along with standard deviation (SD) for the tall and dwarf group, the mean differences between the tall and dwarf group, and the effects of dwarfing gene Rht5 on the agronomic traits in wheat were estimated and showed in Table 4. The highest values for standard deviation in the dwarf group were observed for number of grains spike<sup>1</sup>, and number of fertile tillers plant<sup>1</sup>, which suggested, greater magnitude of variability among dwarf lines for these traits and the potential for selection of good lines with better agronomic traits and semi-dwarf architecture.

The effects of Rht5 on plant height and peduncle length

The plant height and peduncle length of the tall group were taller and longer than that of the dwarf group by 22.4% and 20.4%, respectively, which indicated that the dwarfing gene Rht5 could reduce the plant height by 22.4% in general. Great variations were observed in both tall and dwarf groups, suggesting a great potential for selecting individuals with proper height for wheat production.

The effects of Rht5 on heading date and fertile tillers

The heading date of the tall group was about 3.62 days earlier than that of the dwarf group, which was less than the difference between the two parents. Correspondingly, the number of fertile tillers plant<sup>1</sup> of tall group was less than that of the dwarf group by 29%, which also was less than the difference between two parents. These findings suggested that delaying heading date was one of the drawbacks of dwarfing gene Rht5, but the effects could be reduced during the selection.

The effects of Rht5 on spike characters

The spike length of the tall group was significantly longer than that of the dwarf group by 12.22%. The number of spikelets spike<sup>1</sup> and number of grains spike<sup>1</sup> of the tall group were more than that of the dwarf group by 6.4% and 15.7%, respectively. Compared with the difference between the two parents, the number of grains spike<sup>1</sup> of the dwarf groups could be improved greatly, in general by 23.6% than the dwarf parent.

The effects of Rht5 on biomass, grain yield and harvest index

The analysis on the biomass and grain yield indicated that there were no significant differences between the tall group and dwarf group, although the biomass and grain yield of tall group were a little higher than that of the dwarf group. This resulted in a little less harvest index of the tall group than that of the dwarf group by 3.45%. These findings suggested that the dwarfing gene Rht5 could improve the harvest index.

Correlation between plant height and other traits

Correlation analysis between plant height and other traits (Table 5) revealed that plant height had highly positive and significant correlation with peduncle length, spike length, number of spikelets spike<sup>1</sup>, number of grains spike<sup>1</sup>, whereas it had negative and highly significant correlation with number of fertile tillers plant<sup>1</sup> and heading date. No significant correlations were observed between plant height with grain yield and harvest index.

Discussion

Crop establishment is a major determinant of yield. It was reported by Ellis et al. (2004) that dwarfing gene Rht5 reduces adult plant height without affecting early growth. Peduncle length has also been suggested as a useful indicator of yield capacity in dry environment. This study is carried out to investigate and obtain a better understanding of the effects of dwarfing gene Rht5 on plant height and correlated agronomic traits of common wheat. As expected, Rht5 genotype has a significant effect on plant height. This study found height reduction of 23.91% in F<sub>2</sub> population and a peduncle length reduction of 20.4% in the F<sub>3</sub> lines, therefore contributing to a height reduction of 22.4% by Rht5. An average of 23.16% plant height reduction could be therefore associated with Rht-5. Rebetzke et al. (2012) reported height reductions of 55% due to Rht5. The difference in height reduction could be due to the different genetic backgrounds and environmental conditions. Robbins (2009) reported height reductions of 20.5%, 24.4% and 10.1% due to Rht-B1b, Rht-D1b and Rht8, respectively. Height reduction of 34% by Rht13 was also reported by Rebetzke et al. (2012). The result of this study indicated that Rht5 could approximately have the same height reducing effect with Rht-B1b but stronger effect than Rht8 allele. Heading date is one of the most important traits for wheat performance and improvement because it affects the adaptability of the crop to environmental conditions including water-stress (Law et al., 1998; Law, 1998). There was a general trend for reduced height genotypes to be associated with later maturity across the population. Lines containing Rht5 alleles were later flowering and being particularly slow in development. In this study, we observed that dwarf group headed 10 to 24 and 2 to

<table>
<thead>
<tr>
<th>Genotype Class</th>
<th>Ningchun10(rht5)</th>
<th>Marled M(Rht5)</th>
<th>Difference</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plant height(cm)</td>
<td>140.7±0.68</td>
<td>77.27±0.57</td>
<td>63.48</td>
<td>45.1</td>
</tr>
<tr>
<td>Peduncle length(cm)</td>
<td>48.28±2.05</td>
<td>24.82±1.15</td>
<td>23.46</td>
<td>48.59</td>
</tr>
<tr>
<td>Heading date (d)</td>
<td>207±1.41</td>
<td>215±0.0</td>
<td>-8</td>
<td>-3.86</td>
</tr>
<tr>
<td>No. fertile tillers plant&lt;sup&gt;1&lt;/sup&gt;</td>
<td>14.25±4.24</td>
<td>29.6±7.64</td>
<td>-15.35</td>
<td>-107.71</td>
</tr>
<tr>
<td>Spike length(cm)</td>
<td>13.72±1.44</td>
<td>7.37±0.80</td>
<td>6.35</td>
<td>46.28</td>
</tr>
<tr>
<td>Spikelets No. spike&lt;sup&gt;1&lt;/sup&gt;</td>
<td>21±1.41</td>
<td>18.84±1.65</td>
<td>2.16</td>
<td>10.28</td>
</tr>
<tr>
<td>Grains No. spike&lt;sup&gt;1&lt;/sup&gt;</td>
<td>53.5±8.73</td>
<td>26.8±2.12</td>
<td>26.61</td>
<td>46</td>
</tr>
<tr>
<td>Biomass(t/ha)</td>
<td>28.45±3.32</td>
<td>15.28±7.64</td>
<td>13.11</td>
<td>46.29</td>
</tr>
<tr>
<td>Grain yield(t/ha)</td>
<td>8.57±0.06</td>
<td>3.81±2.02</td>
<td>4.76</td>
<td>55.54</td>
</tr>
<tr>
<td>Harvest index</td>
<td>0.30±0.04</td>
<td>0.2±0.03</td>
<td>0.105</td>
<td>34.43</td>
</tr>
</tbody>
</table>

Difference was calculated by the value of tall parent minus that of the dwarf parent; the percentage was then estimated by percentage of the difference to tall parent.

Table 3. Comparison on the traits investigated between the tall and dwarf parent.
6 days later than the tall group in F2 population and F2:3 lines, respectively; and when compared with the dwarf parent earlier heading date could be observed in dwarf lines. These findings suggested close linkage of Rht5 with alleles for later maturity as reported by Rebetzke et al. (2012), but dwarf lines with earlier maturity could be selected in the progeny. Earlier flowering and maturity in reduced-height lines containing Rht8 was reported by Rebetzke et al. (2012). Robbins (2009), reported little to no effect on heading date associated with Rht1, Rht2 and Rht8. Number of fertile tillers per plant, an important yield component, is playing a vital role in increasing the final grain yield. Semi dwarf wheat cultivars can have more fertile tillers and grain yield than wheat variety with high plant height. In present study, dwarf group with Rht5 yielded more fertile tillers than tall group by 29%, which could be attributed to the mother parental line (Marfed M). Mahboob et al. (2002) reported genotypes having Rht1 gene produced significantly more and fertile tillers plant-1 leading to higher yield per plant. Spike length hold great value for plant breeders as it determines the yield of wheat. The spike length was reduced by 21.16% for the genotypes with Rht5 in the F2 population and 12.22% in the F2:3 lines. The number of spikelets spike-1 and number of grains spike-1 of the tall group in F2:3 lines were more than that of the dwarf group by 6.4% and 15.7%, respectively. Compared with the F2 dwarfs and dwarf parent increase in number of spikelets and number of grains spike-1 could be observed in the dwarf lines, which suggested possible selection of dwarf individuals with more grains spike-1. Little effect (-1%) of Rht8 on grain number per spike and 66% reduction in grain number per spike associated with Rht5 were reported by Rebetzke et al. (2012). Rebetzke et al. (2012) also reported increased grain number per spike of 27%, 19%, 19% and 9% associated with Rht13, Rht4, Rht12 and Rht-B1b, respectively. Increased grain yield in GAR dwarfing lines reflected greater harvest index, biomass or both. In our study, there were no significant differences between grain yield, biomass and harvest index of tall and dwarf groups of dwarfing gene Rht5. Compared with the dwarf parent, more grain yield, and higher harvest index could be observed in the dwarf lines. Contrasting results were reported by Rebetzke et al. (2012) where Rht5 was associated with reduction in grain yield. As previously reported (Robbins, 2009) and in our study, Rht5 affected the grain number per spike and number of fertile tillers. However, the data suggested that the presence of Rht5 may require large populations in initial crosses so as to identify earlier flowering and plant height reduced recombinants. However, additional evaluation of Rht5 in differing environment is needed to elucidate its precise effects on plant height and other agronomic traits. The genotypes along with earlier heading and semi dwarf architecture with good agronomic performance should be selected in the dwarfing lines for the next generation.

Materials and methods

Plant material and experimental layout

One hundred and thirty four plants from an F2 population and forty F2:3 best lines from a cross between Ningchun10 (rht5) and Marfed M (Rht5) along with the two parents were evaluated for their genotypes and agronomic traits. Ningchun10 is a tall wheat cultivar in the dryland spring wheat region and without any known dwarfing genes detected. Marfed M is the mutation with dwarfing gene Rht5, strong winter habit, very small spike and late heading and maturity. The experiment was carried out during the two winter wheat growing seasons of 2010-2011 and 2011-2012 at the experimental farm of Northwest A&F University (Shaanxi, P.R China). The altitude of the area is 525 m and the climate is semi-humid prone to semi-arid with an average annual temperature of 13°C and average annual rain fall of 600 mm. The rainfall in this wheat growth season (October 1, 2011 to End of May, 2012) was 220.3 mm. The F2 individuals were planted in an evenly field, in a row of 1.67 m long with an interval of 25cm between rows and 10 cm within plants. The F2:3 lines along with the two parents were grown in a randomized complete block design with two replications. Each line was sown by single seed dibbler method in 3 rows of 1.67 m long, with an interval of 25 cm between rows and 6.67 cm within plants for each line.

Traits evaluation

Five plants of each line per replication were selected at random and indexed to record the data for quantitative traits. Data on heading date (N), plant height (cm), spike length (cm), number of spikelets spike-1 (N), number of grains spike-1 (N) and number of fertile tillers plant-1 (N) were recorded. At harvest, the above ground biomass of each genotype was weighed to estimate the aboveground dry matter production. The grains of each genotype were threshed out separately and the amount of grain weight per genotype was recorded. Harvest index was calculated, as a ratio of grain weight to aboveground biomass, for each genotype.

Genomic DNA extraction and SSR analysis

Genomic DNA was extracted from the mixed fresh young leaves of 5 individuals for each line using CTAB method (Clark, 1997). Polymerase chain reaction was performed with the SSR marker Barch102 to identify the genotype of each F2:3 lines. The BARC102 primer used was as 5'-GGAGAGGACCTGCTAAAATC-3' and 5'-GGTTTACGACGTGTTGAGA-3' as reported by Ellis et al. (2005). Polymerase chain reaction was performed in a volume of 10 μl in a Peltier Thermal cycler. The reaction mixture contained 10X Taq buffer, 10 pmole of each primer, 25 mM MgCl2, 2.5 mM dNTPs, 1U Taq polymerase and 50–100 ng template DNA. Cycling conditions were as an initial denaturation step of 5 min at 94°C, followed by 37 cycles of 30-sec denaturation at 94°C, 30-sec annealing at 52°C, 30-sec extension at 72°C, and a final extension at 72°C for 10 min. The PCR products were separated on 8% polyacrylamide gels. The gels were run in 1X TBE buffer (0.09 M Tris-borate and 0.002 M EDTA) at 170 V for 3 h. The products were visualized by silver staining using the method described by Bassam et al. (1991).

Statistical analysis

Genotypes of the F2:3 lines were identified based on the presence or absence of the target bands amplified by primer BARC102. Based on the genotypes, the lines were classified into 2 groups as tall (rht5) and dwarf (Rht5). Data recorded for various parameters were statistically analyzed using analysis of variance (ANOVA) procedures as described by Steel et al. (1997); multiple comparisons among groups were
conducted by the least significant difference (LSD) test using SAS 8.1 software (SAS Institute Inc., Cary, NC, USA).

**Table 4.** Means, ranges, standard deviations and mean differences for measured traits between Tall (25) and Dwarf (15) F$_{2}$, lines of Ningchun10/Marfed M and the estimated effects of Rht5.

<table>
<thead>
<tr>
<th>Genotype Class</th>
<th>Tall (rht-5)</th>
<th>Dwarf (Rht-5)</th>
<th>Difference</th>
<th>Effect (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean±SD</td>
<td>Range</td>
<td>Mean±SD</td>
<td>Range</td>
<td></td>
</tr>
<tr>
<td>Plant height(cm)</td>
<td>121.63±9.81</td>
<td>105.24~136.97</td>
<td>94.41±9.21</td>
<td>197.63~104.8</td>
</tr>
<tr>
<td>Peduncle length(cm)</td>
<td>40.92±4.43</td>
<td>30.55~48.55</td>
<td>32.59±2.84</td>
<td>26.87~37.24</td>
</tr>
<tr>
<td>Heading date (d)</td>
<td>206.70±4.12</td>
<td>205~209</td>
<td>210.32±2.71</td>
<td>207~215</td>
</tr>
<tr>
<td>No. fertile tillers plant$^{-1}$</td>
<td>17.10±4.01</td>
<td>11.1~25.38</td>
<td>22.06±3.96</td>
<td>16.68~31.17</td>
</tr>
<tr>
<td>Spike length(cm)</td>
<td>10.47±0.77</td>
<td>9~11.68</td>
<td>9.19±0.57</td>
<td>7.84~10.1</td>
</tr>
<tr>
<td>Spikelets No. spike$^{-1}$</td>
<td>21.18±1.05</td>
<td>19.33~23.67</td>
<td>19.83±1.43</td>
<td>17.17~21.84</td>
</tr>
<tr>
<td>Grains No. spike$^{-1}$</td>
<td>39.32±6.57</td>
<td>28.17~51.84</td>
<td>33.16±6.42</td>
<td>25.5~44.84</td>
</tr>
<tr>
<td>Biomass(t/ha)</td>
<td>23.91±7.23</td>
<td>12.63~38.79</td>
<td>22.80±4.51</td>
<td>15.27~32.19</td>
</tr>
<tr>
<td>Grain yield(t/ha)</td>
<td>6.98±2.57</td>
<td>2.83~14.26</td>
<td>6.78±2.42</td>
<td>4.71~8.94</td>
</tr>
<tr>
<td>Harvest index</td>
<td>0.29±0.05</td>
<td>0.2~0.37</td>
<td>0.30±0.05</td>
<td>0.21~0.37</td>
</tr>
</tbody>
</table>

*Difference was calculated by the value of tall group minus that of the dwarf group; the effect was then estimated by percentage of the difference to tall group.*

**Table 5.** Correlation coefficients between Plant height and other agronomic traits among the F$_{2}$, lines of Ningchun10/Marfed M.

<table>
<thead>
<tr>
<th>Traits</th>
<th>Plant height</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heading date</td>
<td>-0.71**</td>
</tr>
<tr>
<td>Peduncle length</td>
<td>0.86**</td>
</tr>
<tr>
<td>Spike length</td>
<td>0.76**</td>
</tr>
<tr>
<td>No. of fertile tillers plant$^{-1}$</td>
<td>-0.30**</td>
</tr>
<tr>
<td>Spikelets No. spike$^{-1}$</td>
<td>0.56**</td>
</tr>
<tr>
<td>Grains No. spike$^{-1}$</td>
<td>0.42**</td>
</tr>
<tr>
<td>Biomass</td>
<td>0.26*</td>
</tr>
<tr>
<td>Grain yield</td>
<td>0.15</td>
</tr>
<tr>
<td>Harvest index</td>
<td>-0.14</td>
</tr>
</tbody>
</table>

*Differences were considered statistically significant when P < 0.05. The effects of dwarving gene were estimated following the formula, effect = (X$_{tall}$ - X$_{dwarf}$)/X$_{tall}$ X 100%. Phenotypic correlation coefficients between plant height with other agronomic traits were worked out with the help of SPSS 16.0 for windows.*

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**References**


