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Genetic Analysis and Molecular Mapping of Low-tillering Mutants (cul2.b and lnt1.a) in Barley (*Hordeum vulgare* L.)

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ABSTRACT

Plant architecture is governed by the action of meristems. During vegetative development, the shoot apical meristem is responsible for initiating all of the above-ground structures including the nodes, internodes, leaves, axillary meristems and the inflorescence. Five barley mutants with low-tiller have been found, currently including, low number of tillers1 (lnt1.a), absent lower laterals1 (als1), intermedium-b (int-b), uniculm2 (cul2.b), uniculm4 (cul4) and semi brachytic (uzu). Specifically, the cul2.b mutant failed to develop tillers, while the lnt1.a mutant can produce 1-4 tillers. Genetic analysis indicated that two mutant phenotypes were caused by two recessive genes cul2.b and lnt1.a, respectively. In this study, two F_2 populations, 279 individuals derived from Bowman×GSHO 531 and 184 individuals derived from Bowman×GSHO 1984 were developed for mapping the cul2.b and lnt1.a genes using Simple Sequence Repeats (SSR) markers. F_3 populations were created to identify genotypes of F_2 individuals. Ultimately, cul2.b was located between SSR markers GBM1212 and Bmag 0613 on the long arm of chromosome 6H, with distances of 12.7 and 13.2 cm to the two markers, respectively. Another five SSR markers (GBM 1319, GBM 1423, Bmag 0807, Bmag 0378 and Bmag 0003) on chromosome 6H were also found around the cul2 gene, with distances of 19.6, 33.3, 34.1, 71.5 and 80 cm to the cul2.b gene. The lnt1.a gene was positioned 7.8 cm away from GBM 1043 on chromosome 3H. This study narrowed the block of tiller development gene in the cul2 and lnt1 mutant. It is a benefit for further map-based clone of the genes.

Key words: Barley, low tillering, cul2.b, lnt1.a, mapping, simple sequence repeat, mutants, clone, genes

INTRODUCTION

The shoot apical meristem is responsible for initiating all of the above-ground structures including the nodes, internodes, leaves, axillary meristems and the inflorescence (Sussex, 1989; DeCook *et al*., 2006; Shaaban *et al*., 2008). Axillary shoots developed from axillary meristem in barley and other grasses are modified branches which develop at the crown of the plant independently of the primary shoot (Kerstetter and Hake, 1997). They are referred to as tillers (McSteen and Leyser, 2005). As one of the important agronomic traits affecting crop yields (Balouchi *et al*., 2005; Al-Shammary, 2005; Wani *et al*., 2011; Abd El-Kareem and El-Saidy, 2011; Jalata *et al*., 2011), tillering is a special branch characteristic during plant development (Riaz and Chowdhry, 2003; Abdellaoui *et al*., 2007; Cho and Kim, 2010).

Generally, the control of tillering is studied through mutant analysis including analyses on natural mutants with the unknown gene and artificial mutants, such as the RAX gene of *Arabidopsis* (Muller *et al*., 2006), the OsTB1 gene in rice (Takeda *et al*., 2003), the MOC1 gene in rice (Li *et al*., 2003a). Although, the research on tillering mechanism of barley is not as deep as *Arabidopsis* and rice, there have been some barley mutants producing significant fewer tillers, such as low number of tillers1 (lnt1.a), absent lower laterals1 (als1), intermedium-b (int-b), uniculm2 (cul2.b), uniculm4 (cul4) and semi brachytic (uzu) (Babb and Muehlbauer, 2003; Bossinger *et al*., 1992; El-Shazly and El-Mutairi, 2006; Denton and Nwangburuka, 2011; Degewione *et al*., 2011; Ayalneh *et al*., 2012).

The research on barley cul2.b mutants reveals that vegetative axillary meristem formation in cul2.b mutants is not affect but fail to develop tillers (Babb and Muehlbauer, 2003). In addition, inflorescence axillary meristems develop into spikelets but the spikelets at the distal end of the inflorescence have an altered phyllotaxy and some are absent (Babb and Muehlbauer, 2003). The cul2.b gene plays a role in the development of axillary meristems into tillers and involves in controlling proper inflorescence development. It also can affect branching by combining with some of the other genes (Babb and Muehlbauer, 2003). The cul2.b gene has been previously mapped onto chromosome 6(6H) of the barley morphological map. Furthermore, the cul2.b gene has been positioned between RFLP molecular marker ABG458/cMWG679 and KFP128 (Babb and Muehlbauer, 2003).

lnt1.a is a spontaneous mutation that is found in Chikurin Ibaraki 2 and Miho Hadaka hybrid groups and it is controlled by a single recessive gene (Dabbert *et al*., 2010). The mutant products 2-3 tillers basically. The reason may be that the lnt1.a gene affects the initiation of axillary meristems like MONOCULM1 in rice (Li *et al*., 2003b; Schumacher *et al*., 1999; Greb *et al*., 2003) or the outgrowth of axillary buds like floral organ number1 in rice (Moon *et al*., 2006; Clark *et al*., 1997). The lnt1.a gene is placed 5.8 cm distal from GBM1043 and 31.0 cm proximal of Bmag 0013. Expression pattern and sequence analysis between lnt1.a and JuBel2 progenitor verified that the JuBel2 is a candidate gene for lnt1.a (Dabbert *et al*., 2010). In this study, we used Simple Sequence Repeat (SSR) markers to map cul2.b locus and lnt1.a locus, in order to facilitate the map-based cloning of cul2.b and lnt1.a genes.

MATERIALS AND METHODS

Genetic materials: The cul2.b mutation with Bowman genetic background was found in a thermal neutron radiation mutagenesis of Kindred (Shands, 1963). The lnt1.a was a spontaneous mutation (cv. Mitake) in a bulk population from a cross between Chikurin Ibaraki 2 and Miho Hadaka and it was controlled by a single recessive gene (Dabbert *et al*., 2010). A near-isogenic line, GSHO 1984, was developed through seven backcross of the Mitake derived lnt1.a into Bowman (Dabbert *et al*., 2010). Bowman was a commercial two-row barley cultivar where only the central spikelets are fertile and give rise to kernels (Dahleen and Franckowiak, 2007). A list of genetic stocks, known few-tillering genes maps positions and the number of backcrosses into the Bowman genetic background are shown in Table 1 and Fig. 1.

Table 1: Barley genetic stocks

Genetic stocks	Tilling habit	Chromosome location	Backcross to Bowman	Source
Cul2.b	low	6HL	7	GSHO 531
Lnt1.a.	low	3HL	7	GSHO 1984
Bowman	Wild-type	Not applicable	Not applicable	Franckowiak

GSHO 531, GSHO 1984, Bowman were obtained from the USDA-ARS National Small Grain Germplasm Research Facility, Bockelman, ID

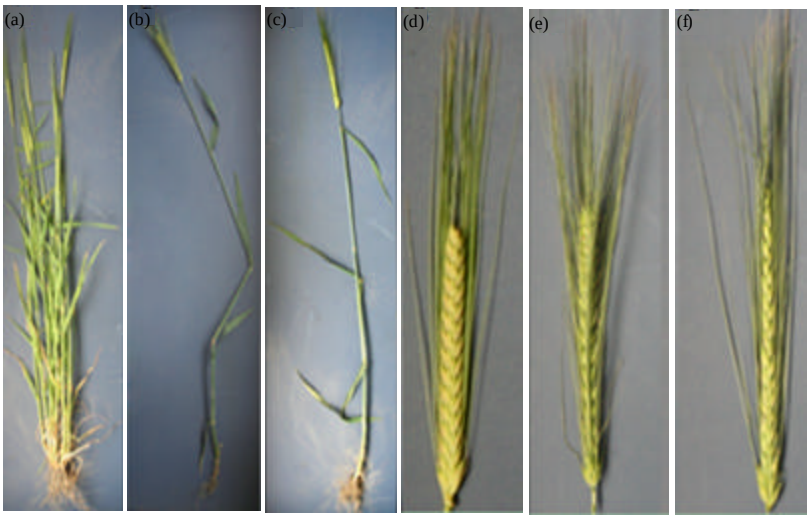

Fig. 1(a-f): Barley of different tiller types, (a-c) Parents vegetative phenotype: (a) Bowman, (b) GSHO 531 and (c) GSHO 1984, (d-f) Barley spikes of parents: (d) Bowman, (e) GSHO 531 and (f) GSHO 1984

Plant population and gene mapping method: To map cul2.b and lnt1.a genes on chromosome, two populations were created including 279 individuals derived from Bowman×GSHO 531 and 184 individuals from Bowman×GSHO 1984. Each line of populations was tested at normal seed density, with classic nitrogen and mineral supply under full fungicide and herbicide protection from Sep. 2008 to Jun. 2010. Segregation ratio in both F_2 populations conformed the law of segregation, resulting in the segregation of two tilling categories including 3/4 multi-tilling and 1/4 low-tilling (Fig. 1). The results demonstrated that the cul2.b and lnt1.a genes were both recessive genes. SSR molecular markers on chromosome 3 and chromosome 6 were used to genotype the low-tilling/multi-tilling F_2 individuals. For each marker, the multi-tilling genotype was recorded as A, low-tilling genotype as B, heterozygote as H and missing data as: Data were analyzed by computer program MAPMAKER/EXP 3.0 (Lincoln *et al*., 1993).

DNA extraction and SSR analysis: Total DNA was extracted from young leaves using SDS method (Gupta *et al*., 2003; Sharp *et al*., 1988). A total of 101 pairs of SSR marker primers were used in the experiment. The sequences of 9 primer pairs were kindly provided by IPK-gatersleben and the rest were obtained from public sources: http://wheat.pw.usda.gov/GG2/index.shtml and in literature (Li *et al*., 2003a; Thiel *et al*., 2003). All of the primers were synthesized by Shanghai Invitrogen Biotechnology Company. SSR analysis was conducted according to previously established protocols with minor modifications (Bryan *et al*., 1997). Each 25 μL PCR reaction mixture consisted of 2.5 μL of 10×PCR buffer (2.5 mM of $MgCl_2$), 0.2 mM dNTPs, 0.3 μmol primers, 100 ng genomic DNA and 1 U *Taq* polymerase. Amplification were carried out in a Gene Amp PCR System 9700 with following PCR program: 5 min of denaturing at 94°C, 45 sec of annealing at 58°C and 1 min of elongation at 72°C. In the following 35 cycles the denaturing time was decrease to 45 sec, with a final elongation step of 5 min at 72°C. PCR products were separated on gels containing 6% polyacrylamide gels and was silver stained (Tixier *et al*., 1997). The poly-acrylamide

gels were scored manually. Linkage analysis was performed using the Map Maker 3.0. Linkage maps were constructed using a LOD threshold of 3.0 and a maximum Kosambi distance of 50.0 cm.

RESULTS

Genetic map location of cul2.b: The cul2.b gene had been previously mapped between RFLP molecular markers ABG458/cMWG679 and KFP128 on to chromosome 6(6H) (Babb and Muehlbauer, 2003; Ruanjaichon *et al*., 2008; Pu *et al*., 2009). According to this information, SSR markers mapping within 10-20 cm of the nearest RFLP marker (KFP128) were chosen to screen Bowman×GSHO 531 F2 population. We firstly detected 279 F2 individuals and 76 of these F2 individuals with multi-tillering, heterozygous and low-tillering genotypes using the marker GBM1212, 67 and 137, respectively. The result showed that the cul2.b gene was located between SSR markers GBM 1212 and Bmag 0613 on the long arm of chromosome 6 H, with distances of 12.7 and 13.2 cm to he two markers, respectively. Another 5 SSR markers (GBM 1319, GBM 1423, Bmag 0807, Bmag 0378, Bmag 0003) on chromosome 6 H were also found around the cul2.b gene, with distances of 19.6, 33.3, 34.1, 71.5 and 80 cm, respectively (Fig. 2).

Genetic map position of lnt1.a: In order to locate the mutant lnt1.a gene, 29 SSR primer pairs were used in this study, the only one (GBM1043) was polymorphic between parents. Among 184 six-row F2 individuals, 106, 36 and 42 individuals showed heterozygous, multi-tillering and

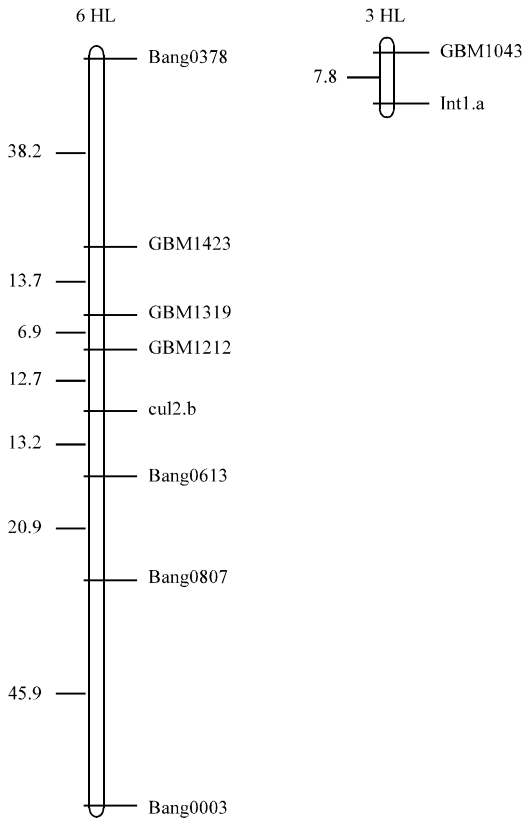

Fig. 2: Map of cul2.b and lnt1.a loci and linked SSR markers

low-tillering genotypes at GBM1043 locus, respectively. Linkage analysis showed that GBM1043 had a distance of 7.8 cm to the lnt1.a. gene (Fig. 2) which was consistent with previous reports by (Dabbert *et al.*, 2010).

DISCUSSION

cul2.b affects plant growth and production of spikelets: The cul2.b allele has been backcrossed into Bowman six times, therefore, morphology differences between few-tillering parents and Bowman can be considered causing by cul2.b. In the farm of Sichuan Agriculture University, the leaves of Bowman×GSHO 531 F_2 population were found curled with folded in leaf edges. Yuan think that a relative long and curled straight leaf with a significant higher light transmission rate is a model for the ideal plant. This type of leaves can improve the base light condition of groups during later period of plant development. Furthermore, it can be found that cul2.b mutants and low-tiller individuals of Bowman×GSHO 531 F_2 population had tall and large culms. Morphology analysis suggested that the development of tillers, leaves and panicle were regulated by a same pathway and gene cul2.b may be a key factor of this approach in cul2.b mutant. Similarly, the negative correlation between tiller number and plant height has been observed in high-tilling dwarf moc1 mutants. This converse regulatory mechanism between cul2.b and moc1 could prompt us to investigate their pathway further.

Morphology of cul2.b and lnt1.a indicate different gene expression patterns: Plant carrying cul2.b fail to grow out axillary meristem and develop into tillers, although normally initiation were not affected (Babb and Muehlbauer, 2003). On the other side, lnt1.a mutation had 1-4 tillers and one or two axillary buds were seen in histological sections of 2 week old seedlings (Dabbert *et al.*, 2009). Additional axillary buds were not observed on the dissected crowns from older lnt1.a. plants, suggesting a block in secondary tiller bud development. Phenotype observation revealed that the two gene (cul2.b and lnt1.a) has different regulation mechanisms and expression periods. In the mutant plant with the both cul2.b and lnt1.a genes, all double-mutants were single tillers but vegetative phenotypes varied. Inflorescence phenotypes of the double mutant were variable when spikes were produced. They ranged from the production of one or a few sterile spikelets to a sterile spike missing spikelets near the distal end of the spike which is similar to that of the cul2.b mutation. It is easy to know that cul2.b was epistatic to lnt1.a.

Conservation among genes regulating branching and tillering: Tomato mutant lateral suppressor (Ls)was isolated by Schumacher, sequence analysis indicated that Ls gene shows 50.5% identity to Ls-homologous gene of *Arabidopsis*, microsynteny studies found that whole genes in this area are conservation whereas the order of genes are different (Greb *et al.*, 2003). Comparison of LAS mutants of tomato and *Arabidopsis* revealed both similarities and differences. Although, both mutants showed a severe reduction in the number of axillary shoots, only the tomato mutant was characterized by a failure to develop petals (Williams, 1960). The Las protein is a member of the GRAS family of putative transcriptional regulators (Pysh *et al.*, 1999), as well as moc1 in rice. The same phenomenon can be found in Bl gene of tomato with R2R3Myb gene of *Arabidopsis*; TB1 gene of maize with BRANCHED gene of *Arabidopsis* (Aguilar-Martanez *et al.*, 2007). Some genes promoted axillary shoot development are homologous to genes repressing tillering and branching, like tomato Ls gene which is homologous to the rice MONOCULM1 gene (Schumacher *et al.*, 1999; Li *et al.*, 2003b). This apparent conservation of genes suggests that genetic pathways controlling

branching and tillering are also conserved, with other functions co-evaluating in plants. There are multiple reviews (Wang and Li, 2006; McSteen and Leyser, 2005; Bennett and Leyser, 2006; Spielmeyer and Richards, 2004) describe the conservation among genes regulating branching and tillering.

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