

Review

The *DENSE AND ERECT PANICLE 1 (DEPI)* gene offering the potential in the breeding of high-yielding rice

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The erect panicle model super-rice can rationally transform the solar energy into accumulated organic matter (biomass) and increase grain yield. The phenotype of erect panicle architecture controlled by *DENSE AND ERECT PANICLE 1 (DEPI)* has been used in rice breeding for nearly a century owing to its high-yield, lodging tolerance with strong stem, reasonable population structure and high nitrogen use efficiency. *DEPI* is a G protein γ subunit that is involved in the regulation of erect panicle, number of grains per panicle, nitrogen uptake, and stress-tolerance through the G protein signal pathway. Here we review the development of erect panicle rice varieties, *DEPI* alleles and regulatory network, and its physiological and morphological functions. Additionally, the further increasing the yield potential of erect-panicle super-rice, and the development of molecular designing breeding for *indica-japonica* hybrid rice with the *depl* gene are also prospected.

Key Words: rice, erect panicle, heterotrimeric G proteins, *DEPI*, high-yield rice.

Introduction

The past century has witnessed two major breakthroughs in rice breeding. The first is the green revolution of the 1960s in which the development of semi-dwarf lines greatly enhanced the rice yield (Peng *et al.* 1999). The second breakthrough is the application of heterosis in hybrid rice, which allowed a continuous increase in rice yield in the 1970s (Virmani *et al.* 1982, Yuan 1998b). Although these two breakthroughs dramatically improved the rice yield, the imminent food crisis that humanity will face in the next 50 years has forced breeders and scientists to search for next breakthrough. To achieve this goal, national and international rice breeding programs have emphasized the improvement of crop productivity by selecting for superior grain yield components and an ideal plant architecture (Huang *et al.* 2009, Khush 1999, Peng *et al.* 2008, Yuan 1998a). Five super rice ideal plant types for different geographical zones in China have been proposed by the Chinese Ministry of Agriculture, which included the erect panicle model that has dominated the northern *japonica* zone (Qian *et al.* 2016). The erect panicle architecture is conferred by a hetero-

meric G protein gene *DENSE AND ERECT PANICLE 1 (DEPI)*, which significantly increases the rice production (Huang *et al.* 2009, Wang *et al.* 2009). Although more than 34 several increasing yield genes have recently been identified in rice (Fan *et al.* 2006, Ikeda *et al.* 2013, Komatsu *et al.* 2003, Li *et al.* 2011, Oikawa and Kyojuka 2009, Shomura *et al.* 2008, Song *et al.* 2007), only *DEPI* has been extensively studied and widely used in rice breeding (Huang *et al.* 2009, Wang *et al.* 2009, Zhou *et al.* 2009).

The objectives of this review are to provide an overview of the *DEPI* function in relation to single plant grain yield and population production, as well as to build a mechanistic model that represents the functions of the *DEPI* gene and its associated pathways. We also discuss the implications of these findings in advancing our knowledge base and in improving rice production.

The history of the erect panicle type

In 1924, ‘Balilla’ was first variety in Italy that was reported to have an erect panicle architecture. This variety was derived from a Chinese *japonica* variety ‘Chinese Originario’, which was introduced from Japan in 1902, and was subsequently used in the generation of a new variety, ‘Ardito’, and later released as ‘Balilla’. Ten rice varieties were introduced into China, including ‘Balilla’ from Italy in 1958, and a number of varieties were bred using ‘Balilla’ as a parent (Xu *et al.* 1995). The profound significance of erect panicle

Communicated by Qian Qian

Received July 28, 2016. Accepted October 19, 2016.

First Published Online in J-STAGE on December 6, 2016.

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architecture was not appreciated until the first widely expanded commercial erect panicle type variety ‘Liaojing 5’ was released in 1976. ‘Liaojing 5’ not only has high yield potential but also has preferable integrated traits, such as lodging resistance and a well-developed vascular system, thereby replacing the predominant Japanese *japonica* in northern China. Shortly afterwards, a number of high-yield *japonica* rice strains with erect panicle were released as commercial varieties (Yang 1984), with several of these meeting the super rice criteria of the Chinese Ministry of Agriculture, including ‘Shennong 265’ and ‘Qinzhongliang2’ (Huang *et al.* 2009, Sun *et al.* 2014). As the erect panicle type varieties began to occupy a predominant place among *japonica* rice in northern China (Chen *et al.* 2001), scientists and breeders began to pay close attention to the erect panicle architecture. Xu *et al.* (1995) earlier reported that the erect panicle trait was controlled by a single dominant gene using the posterity of the cross between ‘Liaojing 5’ and ‘Toyonishiki’ (a popular *japonica* variety). In 2007, a major quantitative trait locus (QTL) that conferred the erect panicle was mapped between DNA markers RM5652 and H90 on chromosome 9 (Yan *et al.* 2007). In 2009, the gene for erect panicle was identified by three independent research teams as *Os09g0441900* and was subsequently named *DEPI/EP/qPE9-1* (Huang *et al.* 2009, Wang *et al.* 2009, Zhou *et al.* 2009). Afterwards, a major rice NUE QTL (*qNGR9*) was cloned from the *japonica* variety ‘Qianzhongliang 2’, which is largely unresponsive to nitrogen fertilization during the vegetative growth period. A subsequent experiment demonstrated that *qNGR9* is synonymous with *DEPI* (Sun *et al.* 2014).

Function of *DEPI/EP/qPE9-1*

The *DEPI* locus is pleiotropic for erect panicle, number of

grains per panicle, nitrogen uptake and metabolism, ABA response and drought tolerance (Huang *et al.* 2009, Sun *et al.* 2014, Zhang *et al.* 2015). *DEPI* has a modular arrangement with a conventional plant-specific $G\gamma$ subunit protein domain at its N-terminus, followed by two von Willebrand factor type C (VWFC) domains, a tumor necrosis factor receptor (TNFR)/nerve growth factor receptor (NGFR) family cysteine-rich domain, and a VWFC domain at the C-terminus. G protein signaling participates in various kinds of growth and developmental processes in animals and plants (Temple and Jones 2007). Heterotrimeric G proteins consisting of three subunits, namely, $G\alpha$, $G\beta$, and $G\gamma$ (New and Wong 1998). The rice genome contains a single $G\alpha$ (*RGAI*) and a single $G\beta$ (*RGBI*) subunits, two canonical $G\gamma$ subunits (*RGG1*, *RGG2*), and three non-canonical $G\gamma$ subunits (*GS3*, *OsGGC2* and *DEPI*) (Botella 2012). The non-canonical $G\gamma$ subunits, which do not occur in mammals, have a conventional $G\gamma$ protein like domain at its N-terminus and a cysteine-rich domain at its C-terminus. A 637-bp stretch in the 5th exon of the dominate *DEPI* locus (*dep1* allele) is replaced by a 12-bp sequence, thereby eliminating the cysteine-rich domain, but not the $G\gamma$ domain. Plant with a *dep1* genetic background that were subjected to RNA interference developed curved panicles and showed a lower number of grains per panicle, whereas those with a *DEPI* genetic background exhibited no obvious changes (Huang *et al.* 2009, Taguchi-Shiobara *et al.* 2011) (Table 1). Similarly, the *dep1*-overexpressing plants presented a dwarf and short panicle phenotype, whereas *DEPI*-overexpressing plants showed no visible changes (Huang *et al.* 2009, Taguchi-Shiobara *et al.* 2011) (Table 1). These experiment evidence demonstrated that *dep1* allele is a gain-of-function mutation. A self-inhibition model was constructed to explain the gain-of function mechanism (Xu *et al.* 2016). The observations that were derived from the *DEPI* mutation and

Table 1. Phenotypes of transgenesis lines of *DEPI* and of NIL lines with different genetic backgrounds

Vector	Receptor	Phenotype	References
<i>RNAi-DEPI</i>	Shao313 (<i>dep1</i>)	Curved panicles and fewer grains	Huang <i>et al.</i> (2009)
<i>pDEPI:dep1</i>	Shao314 (<i>DEPI</i>)	Erect panicle and increased number of grains	
<i>pActin:dep1</i>	Nipponbare (<i>DEPI</i>)	Dwarfed with erect panicles	
<i>pActin:DEPI</i>	Nipponbare (<i>DEPI</i>)	No change	Taguchi-Shiobara <i>et al.</i> (2011)
<i>pActin:Dn1-1</i>	Nipponbare (<i>DEPI</i>)	Dwarfed with erect panicles	
<i>pActin:DN1</i>	Nipponbare (<i>DEPI</i>)	No change	
<i>RNAi-DN1</i>	Nipponbare (<i>DEPI</i>)	No change	
<i>Crispr/Cas9:DEPI</i>	Zhonghua 11 (<i>DEPI</i>)	Dwarfed with erect panicles	Li <i>et al.</i> (2016)

Genetic background	Subspecies	Grain number		Primary branch		Secondary branch		Yield-increasing ^a	References
		<i>DEPI</i>	<i>dep1</i>	<i>DEPI</i>	<i>dep1</i>	<i>DEPI</i>	<i>dep1</i>		
Toyonishiki	<i>japonica</i>	108	146	11	13	16	25	+10.16%	Wang <i>et al.</i> (2009)
Shao314	<i>japonica</i>	130	251	11	16	18	49	+40.90%	Huang <i>et al.</i> (2009)
ZF802	<i>indica</i>	60	290	–	–	–	–	–	Huang <i>et al.</i> (2009)
R6547	<i>indica</i>	303	288	17	17	62	57	–17.20%	Zhou <i>et al.</i> (2009)
RIL- <i>ipa1</i>	<i>japonica</i>	–	–	15	15	63	60	n.s.	Lu <i>et al.</i> (2013)
Zhonghua 11	<i>japonica</i>	104	150	–	–	–	–	–	Li <i>et al.</i> (2016)

“–” indicates the missing data; “n.s.” means no significant change.

^a The increase of yield per plant caused by the *dep1* allele.

another non-canonical $G\gamma$ subunit *GS3* showed that the cysteine-rich domain at the C-terminus has an inhibitory effect on the $G\gamma$ protein like domain at N-terminus (Botella 2012, Mao *et al.* 2010, Xu *et al.* 2016). Replacement of the *dep1* allele resulted in the elimination of the inhibition of the cysteine-rich domain, thereby resulting in an increase of signaling by the $G\beta\gamma$ dimer. The increased $G\beta\gamma$ dimer signal induced two contrasting effects on plant architecture. First, longitudinal cell division and plant height during the vegetative growth period were repressed. Second, cell proliferation and panicle branching during the reproductive stage, which increases meristematic activity and thereby resulting in reduced inflorescence internode lengths (Huang *et al.* 2009, Sun *et al.* 2014). These opposing functions of the *dep1* allele lead to an erect panicle architecture, well developed vascular bundles, an increased number of grains per panicle, and consequently, an increase in the grain yield (Huang *et al.* 2009, Xu *et al.* 2015) (Fig. 1). Because the cysteine-rich region suppressed the $G\beta\gamma$ dimer signal, varia-

tion in the cysteine-rich region caused varying degrees of erect panicle architecture and also changed the dominance, semi-dominance and recessiveness of the alleles (Huang *et al.* 2009, Sun *et al.* 2014, Taguchi-Shiobara *et al.* 2011, Wang *et al.* 2009, Zhou *et al.* 2009). Several studies have proven that the truncated *DEP1* protein (*dep1* allele) increases the number of grains per panicle and subsequently enhance the yield per plant (Huang *et al.* 2009, Wang *et al.* 2009, Xu *et al.* 2014). Recently, the genome editing lines using CRISPR/Cas9 constructs to target *DEP1* in exon 5 also confirmed the function of the truncated DEP1 protein (Li *et al.* 2016). A genetic diversity analysis indicated that a G/C SNP at the promoter region results in a core sequence shift (TGGGCC) for a sit II transcriptional regulatory element, and is significantly associated with the number of primary and secondary branches, as well as number of grains per panicle (Zhao *et al.* 2016). However, Zhou *et al.* (2009) showed that the *dep1/qpe9-1* allele is responsible for the development of an erect panicle architecture and a decrease

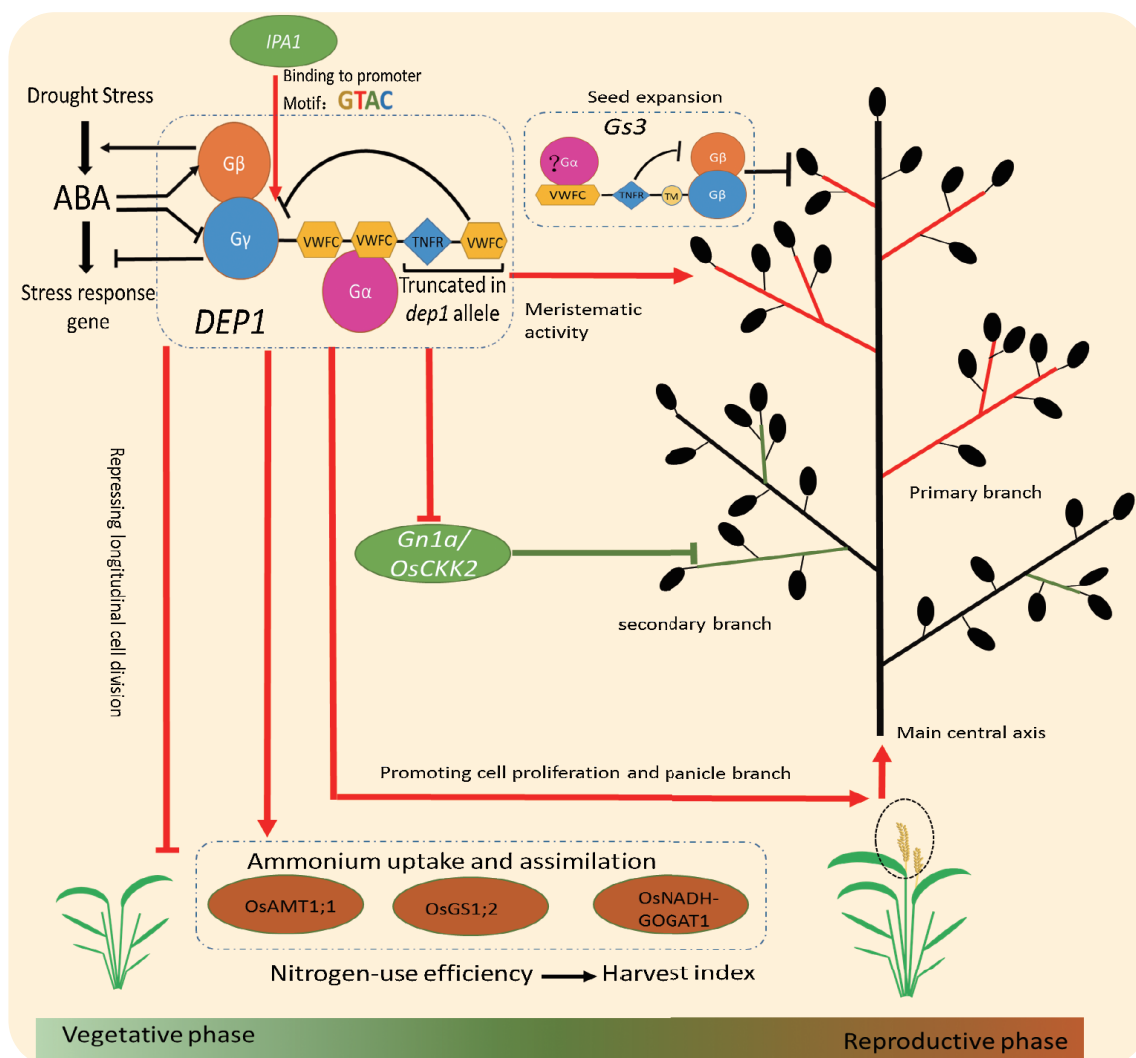


Fig. 1. The *DEP1* network, including the structural characteristics of *DEP1* and *GS3*, the different functions in vegetative and reproductive phases, and upstream and downstream genes. Details are described in the text.

in the number of grains per panicle, which may due to the specific genetic background or cultivation conditions used in the experiment (Zhou *et al.* 2009) (**Table 1**).

The $G\gamma$ protein like domain of *DEP1* strongly interacts with *RGB1*, and the VWFC domain at the C-terminus may participate in association between $G\gamma$ and *Ga*. The *depl* allele inhibits nitrogen responses through interactions with the $G\beta$ and *Ga* subunits, and reduced *Ga* or enhanced $G\beta$ activity inhibits nitrogen responses (Sun *et al.* 2014). A recent study showed that high ABA concentrations decrease the transcription level of *DEP1* and increase the transcription level of *RGB1*. Moreover, *DEP1* negatively regulates the ABA response via suppressing the expression of ABA and stress response related gene, whereas *RGB1* positively regulates ABA biosynthesis through upregulating *NCED* gene expression (Zhang *et al.* 2015). Thus, *DEP1* is involved in ABA response and drought tolerance, and modulated by *RGB1* (**Fig. 1**). *DEP1* is also involved in conferring cadmium tolerance to yeast cells and plants, and its cysteine-rich region influences this activity, whereas the $G\gamma$ domain might not be involved (Kunihiro *et al.* 2013).

The *DEP1* Regulation Network

Plant and panicle architectures are complex traits that are determined by several factors. Although our understanding is limited, the available data allowed us to construct a simple network that summarizes how *DEP1* affects plant and panicle architectures (**Fig. 1**). *IPAI*, which confers the ideal rice plant architecture with reduced tiller numbers and increased grain numbers per panicle, directly binds to the *DEP1* promoter at different sites, and the GTAC core motifs are responsible for *IPAI* binding (Jiao *et al.* 2010, Lu *et al.* 2013, Miura *et al.* 2010). RIL lines, which are derived from a cross between ‘Ri22’, an *ipai*-carrying cultivar (Jiao *et al.* 2010) and ‘Liaogeng5’, an erect panicle variety, demonstrated that *IPAI* functions as a positive regulator of *DEP1* for plant height and panicle length in rice (Lu *et al.* 2013). A study performed using the mixed genetic background of the F_2 populations showed that the effects of *DEP1* associated with *IPAI* binding sites might be obscured or disrupted by different pathways (Xu *et al.* 2014). Thus, a complex relationship may exist between *DEP1* and *IPAI*. *IPAI* also apparently induces two contrasting effects on plant architecture. A higher *IPAI* expression level at the vegetative stage represses shoot branching, thereby leading to a decrease in panicle number. However, a higher *IPAI* expression level in young panicles promotes branching, which increases the number of grains per panicle (Jiao *et al.* 2010). The transition from the vegetative to reproductive phase is marked by the transformation of the shoot apical meristem into an inflorescence meristem. Factors that trigger this functional transition between *DEP1* and *IPAI* in the vegetative and reproductive phases is worth further study. Xu *et al.* (2014) demonstrated that *IPAI* and *DEP1* have contrasting effects on most yield components, and thus the identification of a

balance between *IPAI* and *DEP1* may be another key point that would facilitate in yield improvement.

Gn1a, the first major QTL of yield-related traits is located downstream of *DEP1* (Huang *et al.* 2009). *Gn1a* encodes a cytokinin oxidase/dehydrogenase, which regulates the meristematic activity, panicle branching and grain number through its effects on cytokinin levels (Ashikari *et al.* 2005). The *depl* allele down-regulated the expression of *Gn1a* (Huang *et al.* 2009). A NIL-*Gn1a* line had the same number of primary branches as the control line but developed a higher number of secondary branches (Ashikari *et al.* 2005). This finding suggests that *depl* genetically controls the number of primary and secondary branches at the panicle top, whereas *Gn1a* regulates the number of secondary branches at the panicle base (**Fig. 1**). The restorer line 93-11, which harbors the *depl* and *gn1a* allele, exhibited a remarkable yield performance, reaching as high as 12 t ha⁻¹ (Liu *et al.* 2012). *LAX*, *FZP* and *RCN1* are involved in axillary meristem initiation and development, and *DEP1* rarely affects these genes (Komatsu *et al.* 2003, Nakagawa *et al.* 2002, Oikawa and Kyozuka 2009).

GS3, another non-canonical $G\gamma$ subunit, is a homolog of *DEP1* and was identified a grain size QTL under different genetic backgrounds (Fan *et al.* 2006, Mao *et al.* 2010, Takano-Kai *et al.* 2009). *GS3* has a similar isoform as *DEP1*, where it comprises a $G\gamma$ subunit domain, a transmembrane domain, a TNFR/NGFR family cysteine-rich domain, and a VWFC domain at the C-terminus. The $G\gamma$ domain functions as a negative regulator of grain size and organ size, and the C-terminal TNFR/NGFR domain plays an inhibitory role to the $G\gamma$ subunit domain (Mao *et al.* 2010). Although no experimental evidence as demonstrated the interactions between *DEP1* and *GS3*, these provably function using the same pathway. These may be manipulated simultaneously because these are both $G\gamma$ subunits, and they confer signals through a $G\beta\gamma$ dimer. Erect panicle varieties show a common disadvantage of low 1,000 grain weights, and *GS3* regulates the grain size and weight. Thus, the identification of a factor that simultaneously regulates *DEP1* and *GS3* may improve the yield of erect panicle varieties. Although a number of studies have demonstrated the relationship among *DEP1*, *IPAI* and *Gn1a* (Huang *et al.* 2009, Lu *et al.* 2013, Xu *et al.* 2014), there is a need to elucidate the relationship between *DEP1* and *GS3*.

DEP1 regulates nitrogen uptake and metabolism by affecting genes that are associated with ammonium uptake and assimilation (such as *OsAMT1;1*, *OsGS1;2* and *OsNADH-GOGAT1*) (Huang *et al.* 2009). In *depl* allelic plants, these genes were up-regulated under low nitrogen conditions. The *depl* allelic plants have higher glutamine synthase activities and accumulate higher amounts of internal nitrogen than the *DEP1* allelic plants, regardless of limited nitrogen supply (Sun *et al.* 2014). *DEP1* also regulates ABA responses by affecting the expression of ABA- and stress inducible genes, such as *OsABI5*, *OsRAB16A*, *OsLIP9*, *OsNAC5* and *OsNAC6* (Zhang *et al.* 2015).

Homologs of *DEP1* and other related genes

The homologs of *DEP1* regulate organ size and shape in other species. *AGG3*, a *Gγ* subunit in *Arabidopsis* that has a 22.5% amino acid sequence identity with *DEP1*, regulates petal growth by influencing the cell proliferation period (Chakravorty *et al.* 2011, Li *et al.* 2012). Several homologs of *DEP1* with truncated C-terminal deletions are gain-of-function mutants in barley, bread wheat and its diploid wild progenitor *Triticum urartu* (Huang *et al.* 2009). Because the *Gγ* subunits participate in the regulation of organ size in *Arabidopsis*, and the truncated of *DEP1* homologs are also occur in some cereals, the use of *DEP1* homologs in other crops may contribute to solving the global food crisis.

DEP2, which is allelic to *EP2* (*Os07g0616000*), was identified from mutations of ‘Zhonghua 11’ and ‘Nipponbare’ (Abe *et al.* 2010, Li *et al.* 2010). *DEP3* (*Os06g0677000*) was identified from a mutation in an elite Korean japonica cultivar (Qiao *et al.* 2011), and both *dep3* and the *dep2/ep2* alleles are recessive alleles that cause a dense and erect panicle architecture. Experimental evidence did not indicate whether *DEP2/EP2* or *DEP3* is involved in the G protein complex or affects the nitrogen-use efficiency in rice. Further functional characterization of *DEP2/EP2* and *DEP3* may provide a better understanding of the erect panicle mechanism and improve the genetic diversity of erect panicle germplasm.

The effects of *DEP1* on the morphology and physiology in rice breeding

The advantages of the *dep1* allele not only involve its high meristematic activity and the high nitrogen-use efficiency, but also its improved population canopy structure that is caused by erect panicles (Fig. 2). Before the molecular function of *DEP1* was elucidated, breeders and scientists focused on increasing rice yield by inducing morphological changes that result in erect panicles. The photosynthetic ability of panicles was negligible, but the panicles’ shadows may obstruct the light, thereby preventing it from reaching the leaves. Xu *et al.* (1990) conducted a light intensity investigation by comparing the light intensity in the canopy before and after cutting the panicles, and the results showed that the erect panicle plants were less affected by panicle shadows than the curved panicle plants (Fig. 2A). Moreover, the erect panicle architecture may improve the circulation of CO₂ and moisture (Xu *et al.* 1995) (Fig. 2). A population structure analysis based on field investigation showed that the subsequent opening of the plant canopy through erect panicle architecture allows a 10% increase in sunlight penetration compared to curved panicle varieties (Xu *et al.* 1996). In addition, the canopy temperature of erect type varieties was about 2°C higher than that of curved panicle type varieties during the midday (Xu *et al.* 1996). Wind speed within the canopy is also much faster with erect panicle varieties than with curved panicle varieties, thereby resulting

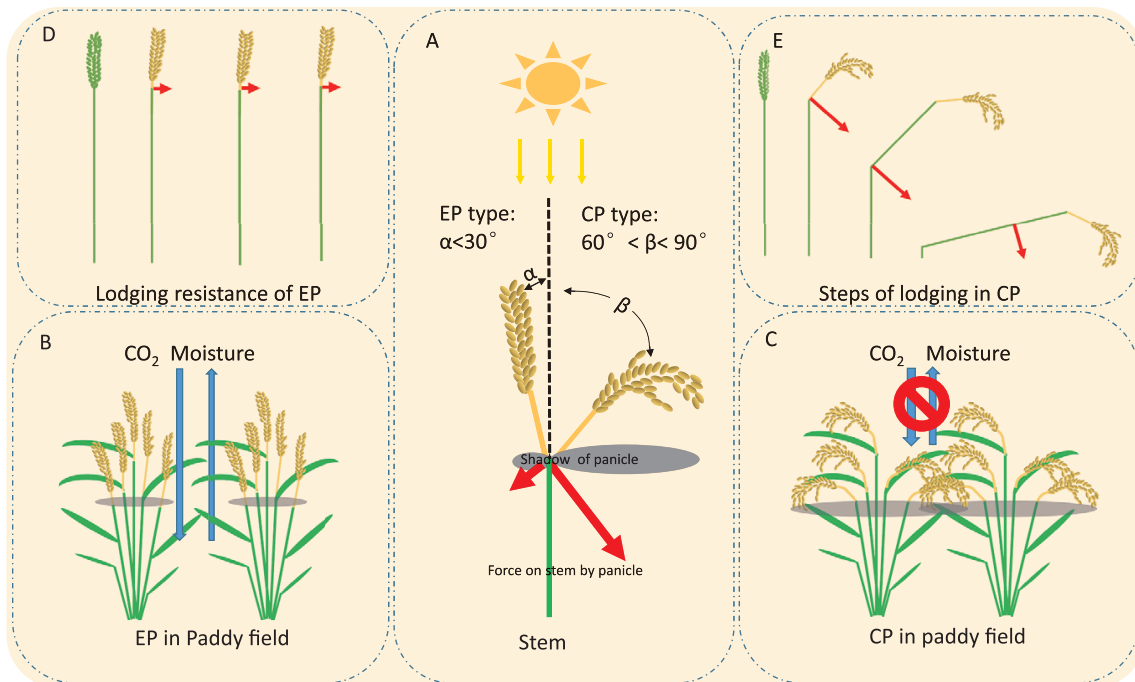


Fig. 2. The effects of *DEP1* on morphology. (A) The panicle shadow on a leaf at noon and the force of the panicle on the stem between erect and curve panicle plants. (B, C) The erect panicle improves the circulation of oxygen and moisture compared to that in the curved panicle. (D, E) The erect panicle decreases the force on the panicle neck and stem, and consequently improves the resistance to lodging compared to that in the curved panicle. EP and CP represent erect panicle architecture and curved panicle architecture, respectively.

in a 6% decrease in canopy humidity using erect panicle varieties compared to that with curved panicle varieties (Xu *et al.* 1996). The relatively low level of humidity may be beneficial to improving tolerance to plant diseases and insect pests, such as sheath blight tolerance. In the curved panicle variety, gravity bends the panicle neck, thereby forcing the upper internode, and ultimately inducing the entire plant to lodge. The erect panicle varieties showed strong lodging resistance (Xu *et al.* 2004b) (Fig. 2). Well development vascular bundles and large diameter stems contribute to lodging resistance, and the erect panicle leads to a smaller panicle neck angle compared to that of the curved type, which decreases the force on the panicle neck and stem, and consequently improves the resistance to lodging (Xu *et al.* 2004b).

Application of *DEP1* in rice production

Unlike most other yield-related genes that were identified through molecular research, *DEP1* has been extensively

used in rice breeding (Qian *et al.* 2016). The 3-years average yield of an erect panicle variety, ‘Shennong 606’, has reached 12.34 t ha⁻¹ (Xu *et al.* 2004a). An allelic investigation of 78 *japonica* rice cultivars that were widely cultivated in northeast China between 1963 and 2008 showed that after the release of ‘Liaojing 5’, the application of the *dep1* allele continued to increase in *japonica* varieties in northern of China (Zhao *et al.* 2016). More than 70% cultivars in Liaoning Province carry the *dep1* allele, and more than 50% of the *dep1* carrying cultivars are propagated in northeast of China. At the downstream region of the Yangtze River, nearly all the *japonica* varieties have erect panicle architecture (Xu *et al.* 2007) (Fig. 3A). The northeast area and downstream region of the Yangtze River represents over 70% of entire *japonica* rice cultivated area in China. Moreover, the cultivation area for erect panicle varieties has annually increased in the *japonica* zone (Fig. 3A). For other yield related genes, the long grain type allele of *GS3* has historically predominated in northern China (Sun *et al.* 2012). A high proportion of the *dep1* and *gn1a* alleles have

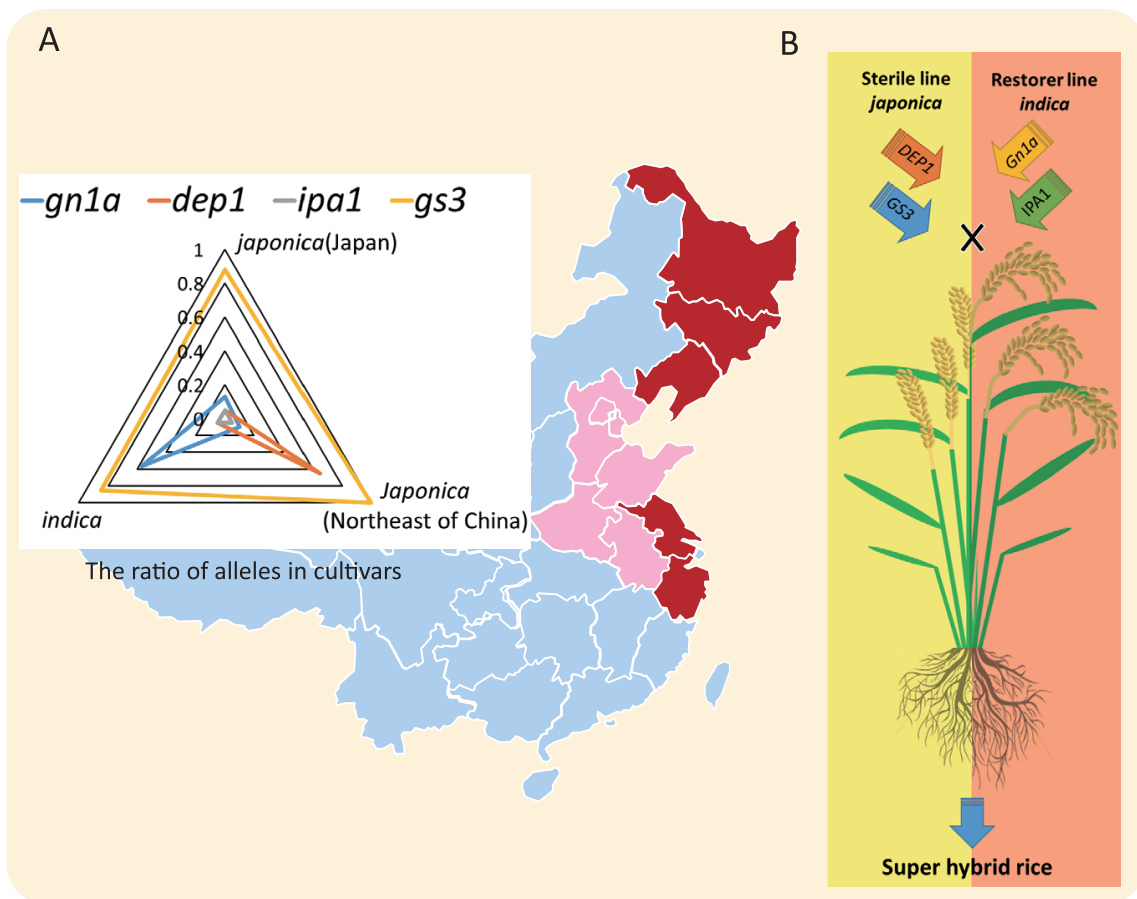


Fig. 3. The application of *DEP1* in rice breeding. (A) Distribution map of erect panicle varieties carrying the *dep1* allele. Regions of large-scale cultivation of erect panicle varieties are indicated in dark red, the area that has generated erect panicle varieties are shown in pink. The top left radar chart shows the allele ratio of *gn1a*, *dep1*, *ipa1*, and *gs3* in *japonica* (separated into *japonica* in Japan and *japonica* in northeast of China) and *indica* varieties (Sun *et al.* 2012). (B) A model for breeding super hybrid rice by rational genetic design using *DEP1* and related genes. The *japonica* sterile plant with the desired alleles of *DEP1* and *GS3*, and the *indica* restorer plant carrying the favorable alleles of *IPA1* and *Gn1a*. Thus, the F₁ hybrids may exhibit an optimized yield components.

been detected in *japonica* (northeast of china) and in *indica* (Fig. 3A), indicating that *dep1* and *gn1a* contribute the rice production improvement in *japonica* and *indica* rice production, respectively. Both high yielding alleles *dep1* and *gn1a* do exist in Japanese *japonica* varieties (Sun *et al.* 2012), which are generally low yielding. We also observed that even though the *ipa1* allele showed an unequaled phenotype for rice production, the *ipa1* allele rarely existed in both *indica* and *japonica* rice varieties (Sun *et al.* 2012).

The bloom of genome information from the global rice germplasm and next generation sequence may facilitate in the breeding of an elite super rice variety using *indica-japonica* heterosis. The *DEP1* also has a potential to contribute to the hybrid super rice breeding by rational design. In the F₂ population derived from the cross between ‘Liaojing 5’ and ‘Ri22’, heterozygous plants showed better yield performance compared to plants that were homozygous at both *DEP1* and *IPAI* loci (Xu *et al.* 2014). Crossing a *japonica* sterile line with a desired allele of *DEP1* and *GS3* with an *indica* restorer line that carried a favorable allele of *Gn1a* and *IPAI* is expected to show an optimal yield components (Liu *et al.* 2012) (Fig. 3B). The F₁ plant with these elite alleles will exhibit an erect panicle architecture, large grains, increase in number of grains at the both top and bottom of the panicle, moderate plant height, and a balanced relationship between the number of grains per panicle and the number of panicles (Chen 2013).

Conclusions and Prospective

The G γ subunit protein, which is encoded by *DEP1* gene, contributes to an increase in grain number, a high nitrogen-use efficiency, and improved drought tolerance. The erect panicle architecture also lead to an improvement in canopy structure and lodging resistance, which in turn benefits rice production (Huang *et al.* 2009, Xu *et al.* 1990, 2004b). Thus, *DEP1* can increase the rice yield, enhance the tolerance to environmental changes, and relieve the pressure of environmental pollution such as excessive application of nitrogenous fertilizers. These elite traits persuade us that *DEP1* may be the key factor that drives improved rice production breakthrough. However, although the findings of the present study represent significant progress in the study of *DEP1* functions, several important pieces of information have yet to be elucidated. As the G protein works very upstream of growth and developmental processes, and G γ subunits affects stomatal movement and lateral roots in *Arabidopsis* (Trusov *et al.* 2007), *DEP1* probably affects other crucial growth processes in rice. As illustrated in Fig. 1, details on the pathways and the network underlying the *DEP1* functions have gradually been uncovered, and the identification of other genes involved in these processes is essential for the characterization of the regulatory network. *DEP1* may regulate cytokinin production through *Gn1a*, as well as the responses to ABA, but whether auxin, strigolactone, brassinolide or other hormones are involved in the

DEP1 network have yet to be established. The introduction of the *dep1* allele in *japonica* breeding makes its absence in *indica* varieties difficult to understand. We believe the *indica* breeding has primarily focused on the heterosis of the F₁ plants in the past decades, while *japonica* breeding has built ideal plant and panicle architectures to achieve higher yields. As the *dep1* allele showed superior yield components under the *indica* genetic background compared to that of the *japonica* background (Xu *et al.* 2015), we believe that the combination of the heterosis in F₁ plants and the *dep1* allele may improve *indica* yield. And rational design of ideal architecture using defined genes, such as *DEP1* and its related genes, will exploit strong *indica-japonica* heterosis along with predetermined specific morphological changes and functional characters. However, erect panicle is not perfect, it showed a low setting rate at the bottom of panicle and are considered to be only mediocre eating quality. Future breeding projects on *DEP1* utilization should address these inadequacies. Because the erect panicle type has a specific morphology and nitrogen response traits, a matching cultivation method that includes the density of plant and the amount of fertilizer, should be developed. Four step identification process for rice breeding were proposed as followings: (i) identification of genes that control traits, (ii) assessment of the characteristics of the particular architecture, (iii) establishment of the population structure, and (iv) population structure that makes the maximum use of the solar energy in given ecological conditions to achieve the yield limits (Zhang *et al.* 2008). The ability to readily incorporate yield improve genes into rice varieties will become increasingly important. Thus, a system for the application of yield-related genes that have been identified through molecular research investigations to rice breeding schemes to maximize yield should be established in the near future.

Acknowledgements

This work was supported by grants from the National Natural Science Foundation of China (No. 31430062, 31501284 and 31371587) and a class general financial grant from the China Postdoctoral Science Foundation (Grant No. 2014M560211, Postdoctoral No. 142541).

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